# Metronidazole Esters: A New Class of Antiglycation Agents

Aurang Zeb,[1] Imran Malik,[1] Saima Rasheed,[1] Muhammad Iqbal Choudhary,[1, 2] and Fatima Z. Basha[*1]

[1]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*

[2]*Department of Chemistry, College of Science, King Saud University, Riyadh-11451, Saudi Arabia*

***This paper is dedicated to Prof. Dr. Atta-ur-Rahman, FRS on the occasion of his 70th birthday***

**Abstract:** A series of metronidazole ester derivatives **1-34** has been synthesized with the aim of developing new leads with antiglycation activity. The *in vitro* evaluation of antiglycation potential of **1-34** showed that the ester derivatives **28**, **16**, and **3** have $IC_{50}$ values 218.97 ± 2.5, 245.3 ± 5.1, and 278.6 ± 0.8 $\mu$M, respectively, comparable to the standard agent, rutin ($IC_{50}$ = 294.5 ± 1.50 $\mu$M). The study identifies a new class of potent antiglycation agents. A structure-activity relationship has also been evaluated. All the compounds were characterized by using spectroscopic techniques, including $^1$H NMR, IR, and EI-MS.

**Keywords:** AGE, Bovin serum albumin, Protein glycation, Inhibitors, Metronidazole, Ester derivatives.

## INTRODUCTION

Glycation is a non-enzymatic reaction between reducing sugars and amino acid residues of proteins; it reversibly forms Schiff bases which are rearranged to form relatively stable Amadori compounds. Further oxidation and rearrangements of Amadori products leads to the advanced glycation end-product (AGE) that induces changes in protein structures and functions and plays an important role in the pathogenesis of late diabetic complications (retinopathy, neuropathy, atherosclerosis, end stage renal diseases, rheumatoid arthritis and neurodegenerative disorders) [1,2]. As the incidence of diabetes worldwide is up from 153 million in 1980 to 347 million in 2008, so is the increase in morbidity and mortality due to diabetic complications [3]. Consequently, there has been considerable recent interest in this area to find molecules that can inhibit or slow down the process of glycation. In our continuing quest to discover potent antiglycation agents, we have synthesized several classes of heterocyclic compounds for their antiglycation potential in *in vitro* and *in vivo* models [4-7]. In this context we were intrigued by the recent report that metronidazole exhibit activity in a rodent diabetic model [8]. Consequently, we investigated metronidazole analogs for their antiglycation activity, and report here the results of that study.

Metronidazole, commonly known as Flagyl, is clinically used against various diseases caused by different protozoans and bacteria [9]. In addition, a number of its derivatives have antibacterial [10-13], antiparasitic [14], antiproliferatic [15], antitrichomonal [16, 17], antifungal [17], and *H. pylori* urease inhibitory activities [18]. Moreover, some derivatives have been used recently as tumor hypoxia imaging agents [19, 20]. Metronidazole has a primary alcohol that serve as a starting point to develop derivatives for structure-activity relationship (SAR). In this study we prepared a series of metronidazole esters **1-34** and evaluated them for their antiglycation properties.

*Address correspondence to this author at the H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan; Tel: 021-99261767, and Cell No. 0341-2279107; Fax: +92-21-4819018-9;
E-mail: bashafz@gmail.com

## Materials and METHODS

### General

Metronidazole and all the aryl acids were used without purification unless otherwise stated. $^1$H NMR Spectra were recorded on Avance Bruker 300, 400 and 500 MHz in $CDCl_3$. Infrared (IR) spectra were obtained on Shimadzu FT-IR 8900 spectrometer. The EI-MS were measured on Finnigan MAT-312, Germany, and JEOL MSRoute JMS 600H, instruments (Japan). TLC Analysis was performed on precoated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). BSA (bovine serum albumin) was purchased from Merck Marker Pvt. Ltd. Rutin, methyl glyoxal (40% aqueous solution), disodium hydrogen phosphate ($Na_2HPO_4$), sodium dihydrogen phosphate ($NaH_2PO_4$) and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (USA).

### Synthesis of Metronidazole Esters

In a typical reaction, metronidazole (1.0 mmole) was dissolved in dichloromethane (20 mL) and aryl acid (1.2 mmol) and DMAP (0.35 mmol) were added at 0$^o$ C. Dicyclohexylcarbodiimide (1.2 mmol) was added after five minutes to the above reaction mixture, and then left on stirring for 24 to 50 h at room temperature depending upon the consumption of starting material, as judged by TLC analysis. The reaction was quenched with 20 mL HCl (0.5 M) and then basified with sat. $NaHCO_3$. The mixture was extracted with dichloromethane, separated, dried with $Na_2SO_4$, and evapo-

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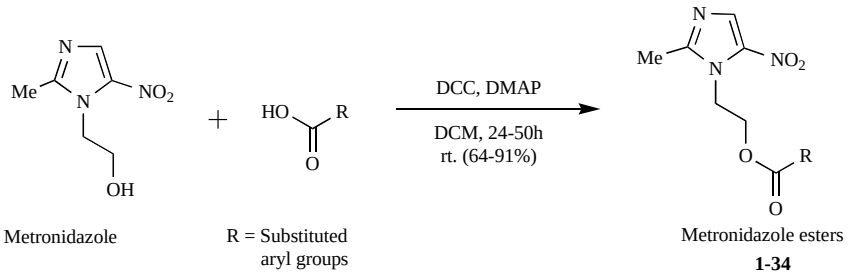



**Scheme I.** Synthesis of metronidazole esters **1**-**34**.

rated *in vaccuo* to give crude product. The mixture of crude product was purified by using silica gel chromatography (EtOAc: hexane, 1/3 to 1/1) (Scheme **I**). The desired ester products **1-34** were obtained in good to excellent yields (64 to 91%). All the compounds were characterized by spectroscopic techniques, including $^1$H-NMR, IR, and EI-MS. Data of the synthesized compounds are described below:

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl benzoate (1)***

Yield: 71%; $R_f$: 0.42 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 3322, 2924, 2848, 1718 (C=O), 1625, 1577, 1532, 1220, 772; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.91 (d, 2H, $J_{2',3'/6',5'}$ = 7.5 Hz, H-2'/H-6'), 7.56 (t, 1H, $J_{4'/3',5'}$ = 7.5 Hz, H-4'), 7.42 (t, 2H, $J_{3',\ 2',4'/5',4',6'}$ = 7.8 Hz, H-3'/H-5'), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 275 ($M^+$, 10), 229 (65), 149 (64), 105 (100), 77 (67).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-methylbenzoate (2)***

Yield: 77%; $R_f$: 0.50 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 2927, 1722 (C=O), 1529, 1465, 1429, 1361, 1253, 1190, 1080, 740; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.70 (d, 1H, $J_{6',5'}$ = 8.1 Hz, H-6'), 7.39 (td, 1H, $J_{4',6'}$ = 1.5 Hz, $J_{4'/3',5'}$ = 9.0 Hz, H-4'), 7.21 (m, 2H, H-3'/H-5'), 4.64 (m, 4H, 2 x $CH_2$), 2.51 (s, 3H, $CH_3$), 2.44 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 289 ($M^+$, 3), 163 (13), 119 (100), 90 (65).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 3-methylbenzoate (3)***

Yield: 91%; $R_f$: 0.46 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 2923, 1722 (C=O), 1529, 1467, 1429, 1361, 1271, 1191, 1107, 744; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.72 (s, 1H, H-2'), 7.68 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6'), 7.36 (d, 1H, $J_{4',5'}$ = 7.5 Hz, H-4'), 7.29 (t, 1H, $J_{5'/4',6'}$ = 7.5 Hz, H-5'), 4.67 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$), 2.37 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 289 ($M^+$, 10), 243 (18), 119 (100), 91 (45).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-methylbenzoate (4)***

Yield: 74%; $R_f$: 0.56 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 2976, 1720 (C=O), 1531, 1469, 1429, 1361, 1267, 1186, 1101, 752; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.78 (d, 2H, $J_{2',3'/6',5'}$ = 8.1 Hz, H-2'/H-6'), 7.21 (d, 2H, $J_{3',2'/5',6'}$ = 8.0 Hz, H-3'/H-5'), 4.66 (m, 4H, 2 x $CH_2$), 2.45 (s, 3H, $CH_3$), 2.38 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 289 ($M^+$, 12), 243 (52), 119 (100), 91 (62), 53 (42).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-bromobenzoate (5)*** [21]

Yield: 85%; $R_f$: 0.45 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 2923, 1732 (C=O), 1529, 1467, 1429, 1361, 1253, 1188, 1134, 746; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.63 (m, 2H, H-4'/H-6'), 7.34 (m, 2H, H-3'/H-5'), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 355 ($M^+$, 4), 353 (4), 185 (47), 183 (48), 56 (100).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 3-bromobenzoate (6)***

Yield: 70%; $R_f$: 0.47 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 3583, 3354, 3099, 1712 (C=O), 1533, 1473, 1361, 1253, 1188, 744; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 8.05 (s, 1H, H-2'), 7.96 (s, 1H, H-4), 7.82 (d, 1H, $J_{6',5'}$ = 7.6 Hz, H-6'), 7.69 (d, 1H, $J_{4',5'}$ = 8.4 Hz, H-4'), 7.30 (t, 1H, $J_{5'/6',4'}$ = 8.0 Hz, H-5'), 4.68 (m, 4H, 2 x $CH_2$), 2.47 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 355 ($M^+$, 5), 353 (5), 229 (20), 227 (20), 185 (97), 183 (100), 53 (48).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-bromobenzoate (7)***

Yield: 68%; $R_f$: 0.44 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 3035, 1732 (C=O), 1525, 1454, 1353, 1263, 1186, 1097, 752; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.76 (d, 2H, $J_{2',3'/6',5'}$ = 8.4 Hz, H-2'/H-6'), 7.57 (d, 2H, $J_{3',2'/5',6'}$ = 8.4 Hz, H-3'/H-5'), 4.67 (m, 4H, 2 x $CH_2$), 2.45 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 355 ($M^+$, 6), 353 (7), 229 (24), 227 (29), 185 (98), 183 (100), 53 (19).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-nitrobenzoate (8)***

Yield: 83%; $R_f$: 0.47 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 3143, 3039, 1732 (C=O), 1614, 1525, 1461, 1344, 1263, 1184, 825, 721; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.95 (s, 1H, H-4), 7.93 (d, 1H, $J_{6',5'}$ = 6.3 Hz, H-6'), 7.67 (m, 3H, H-3'/H-4'/H-5'), 4.66 (br. s, 4H, 2 x $CH_2$), 2.41 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 320 ($M^+$, 17), 274 (22), 194 (100), 150 (100), 134 (85), 76 (30).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 3-nitrobenzoate (9)***

Yield: 70%; $R_f$: 0.44 (EtOAc/hexane 7:3); IR (KBr, cm$^{-1}$): 3143, 3039, 1732 (C=O), 1614, 1525, 1461, 1344, 1263, 1184, 825, 721; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 8.76 (s, 1H, H-2'), 8.43 (dd, 1H, $J_{6',2'}$ = 1.2 Hz, $J_{6',5'}$ = 8.4 Hz, H-6'), 8.22 (d, 1H, $J_{4',5'}$ = 7.8 Hz, H-4'), 7.96 (s, 1H, H-4), 7.65 (t, 1H, $J_{5'/4',6'}$ = 7.8 Hz, H-5'), 4.73 (m, 4H, 2 x $CH_2$), 2.50 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 320 ($M^+$,10), 274 (26), 194 (69), 150 (100), 104 (29).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-nitrobenzoate (10)***

Yield: 86%; $R_f$: 0.49 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3327, 3927, 1714 (C=O), 1624, 1525, 1352, 1271, 1186, 821, 715; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 8.28 (d, 2H, $J_{3',2'/5',6'}$ = 8.7 Hz, H-3'/H-5'), 8.08 (d, 2H, $J_{2',3'/6',5'}$ = 9.0 Hz, H-2'/H-6'), 7.96 (s, 1H, H-4), 4.73 (br.s, 4H, 2 x $CH_2$), 2.48 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 320 ($M^+$, 16), 274 (52), 194 (88), 150 (100), 56(34).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-chlorobenzoate (11)***

Yield: 73%; $R_f$: 0.56 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3131, 2933, 1730 (C=O), 1592, 1532, 1470, 1363, 1292, 1249, 1190, 1052, 748; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.66 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6'), 7.43 (m, 2H, H-3'/H-4'), 7.30 (m, 1H, H-5'), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 309 ($M^+$, 10), 263 (15), 183 (27), 139 (100), 57 (13).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 3-chlorobenzoate (12)***

Yield: 90%; $R_f$: 0.52 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3325, 2927, 1732 (C=O), 1625, 1533, 1473, 1361, 1253, 1190, 744; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.89 (s, 1H, H-2'), 7.77 (d, 1H, $J_{6',5'}$ = 7.73 Hz, H-6'), 7.54 (d, 1H, $J_{4',5'}$ = 7.82 Hz, H-4'), 7.37 (t, 1H, $J_{5'/4',6'}$ = 7.85 Hz, H-5'), 4.68 (m, 4H, 2 x $CH_2$), 2.47 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 309 ($M^+$, 4), 263 (13), 139 (100), 111 (33).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-chlorobenzoate (13)***

Yield: 70%; $R_f$: 0.57 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3325, 2927, 1732 (C=O), 1625, 1533, 1473, 1361, 1253, 1190, 744; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.84 (d, 2H, $J_{2',3'/6',5'}$ = 8.4 Hz, H-2'/H-6'), 7.40 (d, 2H, $J_{3',2'/5',6'}$ = 8.7 Hz, H-3'/H-5'), 4.67 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 309 ($M^+$, 11), 263 (51), 183 (55), 139 (100), 111 (35).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl 2-flourobenzoate (14)***

Yield: 74%; $R_f$: 0.56 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): , 3020, 2934, 2856, 1724 (C=O), 1651, 1613, 1533, 1458, 1364, 1296, 1254, 1191, 1127,1085, 760; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.82 (td, 1H, $J_{6'/2',4'}$ = 1.5 Hz, $J_{6',5'}$ = 7.5 Hz, H-6'), 7.52 (m, 1H, H-4'), 7.19 (t, 1H, $J_{5'/4',6'}$ = 7.8 Hz, H-5'), 7.11 (t, 1H, $J_{3'/2',4'}$ = 9.0 Hz, H-3'), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 293 ($M^+$, 4), 167 (30), 122 (100), 82 (52).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl 3-flourobenzoate (15)***

Yield: 75%; $R_f$: 0.42 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3326, 2925, 1725 (C=O), 1625, 1530, 1467, 1362, 1267, 1191, 755; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.69 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6'), 7.59 (d, 1H, $J_{4',5'}$ = 8.7 Hz, H-4'), 7.41 (m, 1H, H-5'), 7.28 (m, 1H, H-2'), 4.69 (m, 4H, 2 x $CH_2$), 2.47 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 293 ($M^+$, 17), 247 (48), 167 (72), 123 (100), 95 (49).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl 4-flourobenzoate (16)*** [22]

Yield: 85%; $R_f$: 0.42 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3423, 2923, 3099, 1720 (C=O), 1600, 1517, 1456, 1363, 1259, 1182, 1151, 1110, 864, 825, 767; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.91 (m, 2H, H-3'/H-5'), 7.09 (t, 2H, $J_{2',3'/6',5'}$ = 8.4 Hz, H-2'/H-6'), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 293 ($M^+$, 5), 167 (30), 123 (100), 82 (52).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-iodobenzoate (17)***

Yield: 82%; $R_f$: 0.57 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3325, 2927, 1732 (C=O), 1625, 1533, 1473, 1361, 1253, 1190, 744; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.98 (dd, 1H, $J_{6',4'}$ = 0.8 Hz, $J_{6',5'}$ = 7.6 Hz, H-6'), 7.97 (s, 1H, H-4), 7.61 (dd, 1H, $J_{3',5'}$ = 1.6 Hz, $J_{3',4'}$ = 8.0 Hz, H-3'), 7.38 (td, 1H, $J_{4',6'}$ = 1.2 Hz, $J_{4'/3',5'}$ = 7.6 Hz, H-4'), 7.16 (td, 1H, $J_{5',3'}$ = 1.2 Hz, $J_{5'/4',6'}$ = 7.6 Hz, H-5'), 4.69 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 401 ($M^+$, 66), 355 (75), 274 (75), 231 (100), 84 (67).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-vinylbenzoate (18)***

Yield: 80%; $R_f$: 0.49 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 2947, 1720 (C=O), 1606, 1529, 1467, 1429, 1361, 1269, 1186, 1101, 862, 781, 713; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.85 (d, 2H, $J_{2',3'/6',5'}$ = 8.4 Hz, H-2'/H-6'), 7.43 (d, 2H, $J_{3',2'/5',6'}$ = 8.4 Hz, H-3'/H-5'), 6.72 (m, 1H, =CH), 5.85 (d, 1H, $J_{trans\text{-}H}$ = 17.7 Hz, trans H-2"), 5.38 (d, 1H, $J_{cis\text{-}H}$ = 10.8 Hz, cis H-2"), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 301 ($M^+$, 5), 255 (18), 131 (100), 103 (42), 77 (41).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl 4-(bromomethyl)benzoate (19)***

Yield: 81%; $R_f$: 0.42 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3435, 2925, 3099, 1712 (C=O), 1620, 1531, 1469, 1367, 1267, 1188, 1105, 700, 599; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.87 (d, 2H, $J_{2',3'/6',5'}$ = 8.4 Hz, H-2'/H-6'), 7.44 (d, 2H, $J_{3',2'/5',6'}$ = 8.0 Hz, H-3'/H-5'), 4.68 (m, 4H, 2 x $CH_2$), 4.47 (s, 2H, $CH_2Ph$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 369 ($M^+$, 7), 367 (5), 323 (42), 321 (38), 199 (100), 197 (99), 118 (57).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-methoxybenzoate (20)***

Yield: 71%; $R_f$: 0.39 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3741, 2966, 1726 (C=O), 1706, 1529, 1465, 1431, 1361, 1298, 1253, 1188, 1082, 759; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.65 (dd, 1H, $J_{6',5'}$ = 8.1 Hz, $J_{6',4'}$ = 1.8 Hz, H-6'), 7.46 (dt, 1H, $J_{4'/5',3'}$ = 8.7 Hz, $J_{4',6'}$ = 1.8 Hz, H-4'), 6.95 (m, 2H, H-3'/H-5'), 4.67 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 305 ($M^+$, 5), 148 (13), 132 (100), 77 (41).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2,3-diflourobenzoate (21)***

Yield: 79%; $R_f$: 0.47 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3131, 2975, 1729 (C=O), 15 31, 1428, 1363, 1265, 1190, 1147, 1038, 828, 755; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$

7.96 (s, 1H, H-4), 7.58 (td, 1H, $J_{5',3'}$ = 1.8 Hz, $J_{5'/4',6'}$ = 7.8 Hz, H-5'), 7.36 (m, 1H, H-6'), 7.14 (m, 1H, H-4'), 4.70 (s, 4H, 2 x $CH_2$), 2.50 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 312 ($M^+$, 2), 265 (11), 185 (58), 141 (100), 113 (28).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 3,5-diflourobenzoate (22)***

Yield: 79%; $R_f$: 0.49 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3095, 2985, 1732 (C=O), 1598, 1533, 1471, 1328, 1220, 1124, 990, 763, 667; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.40 (dd, 2H, $J_{2',4'/6',4'}$ = 2.1 Hz, $J_{2',3'/6',5'}$ = 7.2 Hz, H-2'/H-6), 7.02 (tt, 1H, $J_{4'/2',6'}$ = 2.4 Hz, $J_{4'/3',5'}$ = 8.4 Hz, H-4'), 4.68 (m, 4H, 2 x $CH_2$), 2.47 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 312 ($M^+$, 6), 265 (17), 185 (40), 141 (100), 113 (57).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2,5-dichlorobenzoate (23)***

Yield: 79%; $R_f$: 0.54 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3440, 2923, 1737 (C=O), 1620, 1525, 1465, 1242, 1147, 1043, 827, 775; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.67 (d, 1H, $J_{6',4'}$ = 2.0, H-6'), 7.38 (m, 2H, H-3'/H-4'), 4.68 (m, 4H, 2 x $CH_2$), 2.47 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 344 ($M^+$, 4), 299 (21), 297 (31), 219 (50), 217 (77), 174 (90), 172 (100), 144 (36).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 3,4-dichlorobenzoate (24)***

Yield: 84%; $R_f$: 0.42 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3423, 2923, 3099, 1716 (C=O), 1529, 1467, 1357, 1263, 1110, 802, 754; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.99 (d, 1H, $J_{2',6'}$ = 1.8 Hz, H-2'), 7.96 (s, 1H, H-4), 7.72 (dd, 1H, $J_{6',5'}$ = 8.4 Hz, $J_{6',2'}$ = 2.1 Hz, H-6'), 7.51 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H-5'), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 344 ($M^+$, 5), 299 (16), 297 (25), 224 (76), 174 (78), 172 (100), 56 (100).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 3,5-dichlorobenzoate (25)***

Yield: 84%; $R_f$: 0.55 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3435, 2923, 1741 (C=O), 1431, 1357, 1259, 1151, 792; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.30 (s, 3H, H-2'/H-4'/H-6'), 4.69 (m, 4H, 2 x $CH_2$), 2.45 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 343 ($M^+$, 10), 299 (6), 297 (8), 219 (29), 217 (45), 175 (85), 173 (100), 147 (12), 145 (20).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2,6-dichlorobenzoate (26)***

Yield: 80%; $R_f$: 0.45 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3442, 3089, 1732 (C=O), 1525, 1361, 1267, 1182, 794, 752; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.77 (d, 2H, $J_{3',5'/5',3'}$ = 2.0 Hz, H-3'/H-5'), 7.55 (t, 1H, $J_{4'/3',5'}$ = 3.6 Hz, H-4'), 4.68 (m, 4H, 2 x $CH_2$), 2.47 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 343 ($M^+$, 10), 216 (45), 172 (100), 144 (19).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-chloro-3-nitrobenzoate (27)***

Yield: 76%; $R_f$: 0.42 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3323, 2923, 1714 (C=O), 1533, 1359, 1255, 1184, 825, 742; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 8.41 (d, 1H, $J_{2',6'}$ = 1.8 Hz, H-2'), 8.01 (dd, 1H, $J_{6',2'}$ = 1.8 Hz, $J_{6',5'}$ = 8.4 Hz, H-6'), 7.96 (s, 1H, H-4), 7.64 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H-5'), 4.70 (br s, 4H, 2 x $CH_2$), 2.48 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 354 ($M^+$, 9), 230 (23), 228 (77), 186 (40), 184 (100), 140 (10), 138 (27), 110 (14), 43 (46).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl 2-chloro-4,5-diflourobenzoate (28)***

Yield: 80%; $R_f$: 0.49 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3437, 3066, 1737 (C=O), 1598, 1531, 1469, 1359, 1234, 1178, 1045, 783; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.60 (m, 1H, H-6'), 7.29 (m, 1H, H-3'), 4.68 (m, 4H, 2 x $CH_2$), 2.46 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 345 ($M^+$, 7), 219 (67), 175 (100), 147 (43), 53 (23).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl 2,4,6-trimethylbenzoate (29)***

Yield: 85%; $R_f$: 0.61 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3436, 2923, 1732 (C=O), 1469, 1261, 1180, 1085, 825; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.95 (s, 1H, H-4), 6.81 (s, 2H, H-3'/H-5'), 4.64 (m, 4H, 2 x $CH_2$), 2.41 (s, 3H, $CH_3$), 2.25 (s, 3H, $CH_3$), 2.13 (s, 6H, 2 x $CH_3$); EI MS *m/z* (rel. abund. %): 317 ($M^+$, 8), 177 (20), 147 (100), 138 (41), 119 (40), 57 (16).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl 2,3,4-trimethoxybenzoate (30)***

Yield: 64%; $R_f$: 0.49 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 2947, 1712 (C=O), 1591, 1483, 1330, 1263, 1132, 999, 759; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.13 (s, 2H, H-5'/H-6'), 4.72 (t, 2H, $J$ = 5.1 Hz, $OCH_2$), 4.63 (t, 2H, $J$ = 5.4 Hz, $NCH_2$), 3.89 (s, 3H, $OCH_3$), 3.86 (s, 6H, 2 x $OCH_3$), 2.48 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 365 ($M^+$, 100), 195 (100), 109 (37), 80 (53), 53 (53).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl thiophene-2-carboxylate (31)***

Yield: 81%; $R_f$: 0.44 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3425, 2923, 1699 (C=O), 1525, 1469, 1427, 1361, 1259, 1186, 1083, 736; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.74 (dd, 1H, $J_{3',4'}$ = 4.0 Hz, $J_{3',5'}$ = 1.2 Hz, H-3'), 7.57 (dd, 1H, $J_{5',4'}$ = 4.8 Hz, $J_{5',3'}$ = 0.8 Hz, H-5'), 7.09 (dd, 1H, $J_{4',5'}$ = 4.8 Hz, $J_{4',3'}$ = 1.2 Hz, H-4'), 4.68 (m, 4H, 2 x $CH_2$), 2.51 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 281 ($M^+$, 30), 235 (88), 155 (75), 111 (100), 53 (14).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl furan-3-carboxylate (32)***

Yield: 79%; $R_f$: 0.42 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3109, 2923, 1722 (C=O), 1527, 1465, 1433, 1361, 1261, 1186, 1159, 756; $^1$H NMR: (500 MHz, $CDCl_3$): $\delta$ 7.96 (s, 1H, H-4), 7.93 (br s, 1H, H-2'), 7.41 (t, 1H, $J_{5',4'}$ = 3.5 Hz, $J_{5',2'}$ = 2.0 Hz, H-5'), 6.63 (d, 1H, $J_{4',5'}$ = 2.0 Hz, H-4'), 4.66 (t, 2H, $J$ = 3.2 Hz, $OCH_2$), 4.58 (t, 2H, $J$ = 3.2 Hz, $NCH_2$), 2.47 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. %): 265 ($M^+$, 5), 139 (23), 95 (100), 53 (12).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl furan-2-carboxylate (33)***

Yield: 72%; $R_f$: 0.40 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 3109, 2923, 1722 (C=O), 1527, 1465, 1433, 1361, 1261, 1186, 1159, 756; $^1$H NMR: (400 MHz, $CDCl_3$): $\delta$ 7.96

(s, 1H, H-4), 7.56 (br s, 1H, H-3'), 7.14 (d, 1H, $J_{5',4'}$ = 4.0 Hz, H-5'), 6.50 (dd, 1H, $J_{4',3'}$ = 3.6 Hz, $J_{4',5'}$ = 2.0 Hz, H-4'), 4.68 (t, 2H, $J$ = 3.2 Hz, $OCH_2$), 4.63 (t, 2H, $J$ = 3.2 Hz, $NCH_2$), 2.52 (s, 3H, $CH_3$); EI MS *m/z* (rel. abund. *%*): 265 ($M^+$, 2), 139 (19), 95 (100), 53 (12).

***2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethyl 1-naphthoate (34)***

Yield: 88%; $R_f$: 0.57 (EtOAc/hexane 7:3); IR (KBr, $cm^{-1}$): 2947, 1712 (C=O), 1591, 1531, 1483, 1330, 1263, 1188, 1132, 999, 759; $^1$H NMR: (300 MHz, $CDCl_3$): δ 8.74 (d, 1H, $J_{2',3'}$ = 8.4 Hz, H-2'), 8.01 (m, 3H, H-4/H-8'/H-7'), 7.87 (d, 1H, $J_{4',3'}$ = 7.5 Hz, H-4'), 7.55 (m, 3H, H-3'/H-6'/H-5'), 4.75 (m, 4H, 2 x $CH_2$), 2.44 (s, 3H, $CH_3$); EI MS m/z (rel. abund. %): 325 ($M^+$, 59), 297 (33), 172 (59), 155 (100), 127 (99).

### *In Vitro* Antiglycation Assay

This assay was carried out to evaluate the potential of esters **1-34** to inhibit the methyl glyoxal mediated development of glycated bovine serum albumin (BSA). In the modified procedure [6], triplicate samples of 10 mg/mL bovine serum albumin (BSA), 14 mM methyl glyoxyal, 0.1 M phosphate buffer (pH=7.4) containing sodium azide (30 mM) was incubated for 9 days under aseptic conditions in such a way that each well of 96-well plate contain 50 $\mu$L BSA solution, 50 $\mu$L methyl glyoxal, and 20 $\mu$L test compound, in the presence or absence of different concentrations of the desired test compounds at 37$^o$ C. After 9 days, the development of specific fluorescence (excitation, 330 nm; emission, 440 nm) was examined for each sample. Rutin ($IC_{50}$ = 294 $\mu$M ± 1.50 SEM) was taken as a positive control. Microtitre plate spectrophotometer (Spectra Max, Molecular Devices, CA, USA) was used to examine the development of fluorescence.

For each potential inhibitor, the percent inhibition of AGE formation was calculated by using the following formula [6]:

% Inhibition ¼ = (1-fluorescence of test sample/fluorescence of the control group) × 100

## RESULTS AND DISCUSSION

The metronidazole ester derivatives **1-34** were synthesized in good to excellent yields (64 to 91%) by reacting metronidazole with different aryl acids in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in dichloromethane at room temperature (Scheme **I**). All the compounds were obtained as solid and easily separated by using silica gel column chromatography.

The structures of all the compounds were determined by spectroscopic techniques, including $^1$H NMR, IR, and EI MS.

Metronidazole and its derivatives **1-34** were subjected to antiglycation assay. This is the first report of antiglycation activity regarding metronidazole ester derivatives. Metronidazole ($IC_{50}$ = 521.60 ± 9.4 $\mu$M) and its ester derivatives **1-34** showed a varying degree of antiglycation activity, with $IC_{50}$ values between 218.97-953.42 $\mu$M (Table **1**). A comparison of these results with the standard rutin ($IC_{50}$ = 294.46 ± 1.50 $\mu$M) showed that esters **28** ($IC_{50}$ = 218.97 ± 2.5 $\mu$M), **16** ($IC_{50}$ = 245.32 ± 5.1 $\mu$M), and **3** ($IC_{50}$ = 278.63 ± 0.8 $\mu$M) have potentially high antiglycation activity. Esters **31** ($IC_{50}$ = 316.73 ± 5.4 $\mu$M), **19** ($IC_{50}$ = 337.06 ± 4.8 $\mu$M), **29** ($IC_{50}$ = 369.48 ± 3.7 $\mu$M), **23** ($IC_{50}$ = 378.09 ± 1.9 $\mu$M), and **5** ($IC_{50}$ = 388.50 ± 1.1 $\mu$M) exhibited a moderate antiglycation potential.

On the other hand, metronidazole (521.60 ± 9.4 $\mu$M) and compounds **18**, **2**, **20**, **30**, **4**, **8**, **12**, **6**, **21**, **15** and **14** showed weak antiglycation activity. The remaining esters **1**, **7**, **9-11**, **13**, **17**, **22**, **24-27** and **32-34** exhibited below 50% inhibition and therefore were not further evaluated for their $IC_{50}$ values.

Compounds **28**, **16** and **3** are the most active analogs of the series with $IC_{50}$ values of 218.97 ± 2.5 $\mu$M, 245.32 ± 5.1 $\mu$M, and 278.63 ± 0.8 $\mu$M, respectively. Compound **28**, with two flouro and one chloro groups on the phenyl ring, is the most active member of the series. Similarly compound **16**, with *p*-flouro on the phenyl ring, also showed a high antiglycation activity as compared to the standard rutin. It appears that the substitution of the *p*-flouro group in this series contributes in the high antiglycation activity, as it can be seen in the other flouro containing analogs i.e. **14** (*o*-flouro), **15** (*m*-flouro), **21** (2, 3-diflouro) and **22** (3, 5-diflouro). In addition, the *p*-flouro substituted analogs also exhibited higher antiglycation potential as compared to other halogen (i.e. Cl, Br and I) containing analogs (**5-7**, **11-13**, **17** and **24-27**).

Compound **3** (*m*-methyl analog), was found to be the third most active analog of the series. The position of Me-group on phenyl ring apparently plays an important role in the antiglycation potential, as it can be seen in the other methyl analogs i.e. **2** (*o*-methyl) and **4** (*p*-methyl) which exhibited lower antiglycation potential. This may be due to their weak interaction with methylglyoxal.

Compound **31** is the fourth most active antiglycating agent. Its potentially high antiglycation activity ($IC_{50}$ = 316.73 ± 5.4 $\mu$M), comparable to the standard, may be attributed to the interaction of sulfur with the carbonyl group of methylglyoxal.

Compound **19** ($IC_{50}$ = 337.06 ± 4.8 $\mu$M), the fifth most active antiglycating agent in the series, is suggested to be active because of the methylbromo substituent at *p*-position of phenyl ring.

Compound **29** (2, 4, 6-trimethyl analog), and compound **18**, with vinyl group on the *p*-position of the phenyl ring, showed a measurable amount of antiglycation potential. This may be due to the electron-donating effects of methyl or vinyl groups.

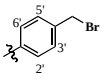
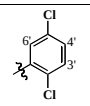
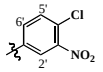
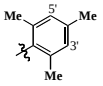
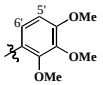
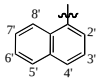
Compounds **20** (*o*-methoxy analog), and **30** (2, 3, 4-trimethoxy analog), showed a sharp decline in activity. The reason may be the steric hindrance caused by these groups during interaction with carbonyl group of methylglyoxal.

Esters **1**, **32-34** possess no inhibitory functional group, capable of reacting with methylglyoxal. They exhibited a complete lack of antiglycation potential.

## CONCLUSION

In conclusion, a total of 34 metronidazole esters were synthesized for antiglycation activity. All target com-

**Table 1. Synthesis of Metronidazole Esters and *in vitro* Antiglycation Activity of Metronidazole and its Ester Analogs 1-34**

| Entry | R | Yield (%) | $IC_{50}$±SEM[a] (µM) | Entry | R | Yield (%) | $IC_{50}$ ± SEM[a] (µM) |
|---|---|---|---|---|---|---|---|
| **1** |  | 71 | Inactive | **18** |  | 80 | 490.16 ± 3.4 |
| **2** |  | 77 | 527.45 ± 4.3 | **19** |  | 81 | 337.06 ± 4.8 |
| **3** |  | 91 | 278.63 ± 0.8 | **20** |  | 71 | 645.14 ± 5.5 |
| **4** |  | 74 | 712.68 ± 2.2 | **21** |  | 69 | 834.82 ± 1.1 |
| **5** |  | 85 | 388.50 ± 1.1 | **22** |  | 70 | Inactive |
| **6** |  | 70 | 819.07 ± 7.5 | **23** |  | 79 | 378.09 ± 1.9 |
| **7** |  | 68 | Inactive | **24** |  | 84 | Inactive |
| **8** |  | 83 | 741.61 ± 7.0 | **25** |  | 84 | Inactive |
| **9** |  | 70 | Inactive | **26** |  | 80 | Inactive |
| **10** |  | 86 | Inactive | **27** |  | 76 | Inactive |
| **11** |  | 73 | Inactive | **28** |  | 80 | 218.97 ± 2.5 |
| **12** |  | 90 | 761.42 ± 1.4 | **29** |  | 85 | 369.48 ± 3.7 |
| **13** |  | 70 | Inactive | **30** |  | 64 | 676.72 ± 6.8 |
| **14** |  | 74 | 953.42 ± 3.2 | **31** |  | 81 | 316.73 ± 5.4 |
| **15** |  | 75 | 951.79 ± 5.4 | **32** |  | 79 | Inactive |
| **16** |  | 85 | 245.32 ± 5.1 | **33** |  | 72 | Inactive |
| **17** |  | 82 | Inactive | **34** |  | 88 | Inactive |
| Metronidazole | | - | 521.60 ± 9.4 | Rutin[b] | | - | 294.50 ± 1.5 |

SEM[a]: standard error of the mean, Rutin[b]: standard inhibitor for antiglycation activity

pounds were structurally identified through spectroscopic methods. The biological evaluation studies indicated that three of these new esters were more potent than the standard. These new compounds could serve as a starting point for further studies in this area.

## CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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