Synthesis, molecular docking, antimicrobial, antiquorum-sensing and antiproliferative activities of new series of pyrazolo[3,4-b]pyridine analogs

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Molecular docking

The 2D structures of compounds were built and converted into 3D using vLife MDS 3.0 software. The 3D structures were then energetically minimized up to the rms gradient of 0.01 using the CHARMM22 force field. All conformers were then energetically minimized up to the rms gradient of 0.01. Molegro 2.5 software\textsuperscript{[1]} was used for molecular docking studies and Lead IT 2.3.2 software\textsuperscript{[2]} was used to generate 2D binding poses. LigandScout 4.1 software\textsuperscript{[3]} was used to generate 3D and 2D pharmacophoric maps. The 3D and 2D pharmacophoric maps for the structural features of 2a are displayed in Supplementary Figure S1A,B. The overlay of pharmacophoric map of 2a to pharmacophoric map of the binding of etoposide to TOP II\textbeta{} is shown in Supplementary Figure S1C.

Computational studies

Computational studies are useful in the evaluation of physicochemical properties, pharmacokinetics and toxicity of compounds\textsuperscript{[4]}. Lipophilicity and aqueous solubility are the principal properties that affect drug absorption. Thus, the new analogs were studied for the prognosis of Lipinski’s rule of five\textsuperscript{[5]} and Veber's criteria\textsuperscript{[6]} using molinspiration software\textsuperscript{[7]}. Also, their carcinogenic properties\textsuperscript{[8]} and drug score values were calculated\textsuperscript{[9]}.
Molinspiration calculations

Lipinski’s rule is valuable in the prognosis of oral absorption of drugs,[5] and it relies on the physicochemical properties of the investigated compounds. Also, topological polar surface area (TPSA) and number of rotatable bonds (Nrotb) affect drug absorption.[6]

Molinspiration software[7] was utilized for studying TPSA, Nrotb and the parameters of Lipinski’s rule of the new derivatives. Results (Supplementary Table S1) articulated that all of the analyzed compounds are under the acceptable norms of Lipinski’s rule, TPSA and Nrotb.

Prediction of carcinogenicity

PreADMET software[8] was used for prediction of possible carcinogenic effect in mice and rats. Data presented in Supplementary Table S2 demonstrated that all of the new analogs are anticipated to be non-carcinogenic in mice. Also, they are expected to be non-carcinogenic in rats (except 2c-f and 3c).

Drug score calculations

The drug score combines drug-likeness, miLogP, solubility, molecular weight, and toxicity risks in one handy value that may be used to judge the compound’s overall potential to qualify for a drug.[10] A drug score value of 0.5 or more makes the compound a promising lead for future development of safe and efficient drugs.[10] The overall drug score values for the synthesized compounds were calculated and compared to that of the standard drugs, ampicillin, fluconazole and doxorubicin using Molsoft software.[9] All of the analyzed compounds possess good drug score values (Supplementary Table S2).

Experimental

Chemistry

Synthesis of ortho aminonitriles 1a-c[11]

A solution of 5-methyl-2,4-dihydro-3H-pyrazol-3-one[12] (0.98 g, 0.01 mol), arylidenemalononitrile[13] (0.01 mol) and ammonium acetate (0.98 g, excess) in ethanol (15 mL) was refluxed for 5-7 h. The solvent was evaporated and the remaining solids were crystallized from ethanol to give compounds 1a-c.

6-Amino-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (1a)

Yield 85%, m.p. 251-252 °C. IR: 3420, 3356, 3267 (NH2, 2NH), 2226 (C≡N). 1H-NMR δ: 1.91 (s, 3H, CH3), 4.76 (s, 1H, C4-H), 5.48 (s, 2H, NH2), 7.27-745 (m, 5H, Ar-H), 10.86 (s, 1H, NH), 12.51 (s, 1H, 1NH). 13C-NMR δ: 11.8, 26.7, 58.9, 103.0, 117.3, 124.7, 127.8, 129.5, 136.1, 137.7, 156.2, 172.3. Anal. Calcd (Found) for C14H13N5: C, 66.92 (66.77); H, 5.21 (5.47); N, 27.87 (27.65)%.
6-Amino-4-(4-chlorophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (1b)
Yield 70%, m.p. 278-279 °C. IR: 3420, 3344, 3287 (NH2, 2NH), 2197 (C≡N). 1H-NMR δ: 2.01 (s, 3H, CH3), 4.95 (s, 1H, C4-H), 6.21 (s, 2H, NH2), 7.47 (d, 2H, Ar-H, J = 7.0 Hz), 7.71 (d, 2H, Ar-H, J = 7.0 Hz), 10.11 (s, 1H, NH). 13C-NMR δ: 28.8, 32.9, 103.9, 116.8, 126.1, 130.6, 132.1, 133.7, 138.2, 155.7, 171.6. Anal. Calcd (Found) for C14H12ClN5 (285.74): C, 58.85 (58.64); H, 4.23 (4.47); N, 24.51 (24.73)%.

6-Amino-4-(4-(dimethylamino)phenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-5-carbonitrile (1c)
Yield 80%, m.p. 226-228 °C. IR: 3397, 3325 (NH2, 2NH), 2207 (C≡N). 1H-NMR δ: 1.93 (s, 3H, CH3), 4.92 (s, 1H, C4-H), 5.87 (s, 2H, NH2), 6.62 (d, 2H, Ar-H, J = 7.5 Hz), 7.23 (d, 2H, Ar-H, J = 7.5 Hz), 9.78 (s, 1H, NH), 11.53 (s, 1H, 1NH). 13C-NMR δ: 12.1, 27.1, 41.6, 59.3, 104.3, 112.9, 118.6, 124.6, 129.9, 137.8, 148.2, 157.2, 173.7. Anal. Calcd (Found) for C16H18N6 (294.36): C, 65.29 (65.47); H, 6.16 (6.32); N, 28.55 (28.83)%.

Synthesis of ethyl N-(heteroaryl)formimidate
A mixture of heteroarlamine (0.01 mol) in triethyl orthoformate (10 mL) was refluxed for 12-16 h. The solvent was evaporated and the remaining residue was triturated with ice, filtered and crystallized from ethanol to give the ethyl N-(heteroaryl)formimidates.

Ethyl N-(pyridin-2-yl)formimidate
Yield 72%, m.p. 96-97 °C. 1H-NMR δ: 1.33 (t, 3H, CH2CH3, J = 7.5 Hz), 3.67-3.70 (q, 2H, CH2CH3, J = 7.5 Hz), 7.42-8.12 (m, 4H, Ar-H), 8.32 (s, 1H, CH=N). 13C-NMR δ: 15.1, 63.7, 116.2, 119.5, 139.3, 148.2, 158.1, 167.6. Anal. Calcd (Found) for C9H10N2O (150.18): C, 63.98 (64.17); H, 6.71 (6.98); N, 18.65 (18.43)%.

Ethyl N-(3-chloropyridin-2-yl)formimidate
Yield 77%, m.p. 112-113 °C. 1H-NMR δ: 1.27 (t, 3H, CH2CH3, J = 7.5 Hz), 3.58-3.61 (q, 2H, CH2CH3, J = 7.5 Hz), 7.49-8.15 (m, 3H, Ar-H), 8.32 (s, 1H, CH=N). 13C-NMR δ: 14.7, 63.7, 122.4, 124.6, 138.5, 146.8, 165.6, 171.1. Anal. Calcd (Found) for C8H9ClN2O (184.62): C, 52.05 (52.37); H, 4.91 (5.22); N, 15.17 (15.33)%.

Ethyl N-(4-phenylthiazol-2-yl)formimidate
Yield 85%, m.p. 163-164 °C. 1H-NMR δ: 1.30 (t, 3H, CH2CH3, J = 7.5 Hz), 3.69-3.72 (q, 2H, CH2CH3, J = 7.5 Hz), 7.39-7.85 (m, 5H, Ar-H), 7.99 (s, 1H, Thiazole-H), 8.11 (s, 1H, CH=N). 13C-NMR δ: 15.0, 64.2, 111.2, 128.4, 128.8, 129.5, 134.0, 152.3, 158.4, 171.7. Anal. Calcd (Found) for C12H12N2OS (232.30): C, 62.05 (61.87); H, 5.21 (5.47); N, 12.06 (12.35)%.

Synthesis of benzamide analogs 2a-f
A mixture of ortho aminonitrile 1a-c (0.002 mol) and benzoyl chloride (0.281 g, 0.002 mol) or 4-nitrobenzoyl chloride (0.371 g, 0.002 mol) was refluxed in glacial acetic acid (10 mL) for 10-16 h. The solution was concentrated and the solid formed was filtered and crystallized from ethanol/water (2:1) to produce 2a-f.
Synthesis of \(N\)-(5-cyano-3-methyl-4-((un)substituted phenyl)-4,7-dihydro-1\(H\)-pyrazolo[3,4-\(b\)]pyridin-6-yl)-\(N\)'-(pyridin-2-yl)formimidamides 3a-e

A mixture of 1a-c (0.002 mol) and ethyl \(N\)-(heteroaryl)formimidate (0.002 mol) in glacial acetic acid (10 mL) was refluxed for 18-24 h. The solution was cooled and the precipitate formed was filtered and crystallized from acetic acid.

**Biological screening**

**Antimicrobial and antiquorum-sensing evaluation**

**Materials**

*S. aureus, B. cereus, E. coli, P. aeruginosa* and *C. albicans* were acquired from Department of Microbiology, Faculty of Pharmacy, Mansoura University, Egypt. *A. fumigatus* 293 was kindly provided by Prof. Nancy Keller, Department of Medical Microbiology and Immunology, Wisconsin-Madison University, USA. *C. violaceum* ATCC 12472 was supplied by Prof. Bob Mclean, Department of Biology, Texas State University, USA.

**Antibacterial assay**

**Determination of diameter of inhibition zone (mm)**

All the bacterial strains were propagated in Luria Bertani (LB) broth (1% peptone, 0.5% yeast extract, 0.5% NaCl) and solidified with 1.5% agar. Melted Muller Hinton agar (50 mL) at 50 °C were seeded with 50 µL of 1x10^8 CFU/mL of 18 h culture of the tested bacteria. The inoculated agar was mixed and poured into 15-cm-diameter plates to solidify. Wells were made in agar using cork borer. Tested compounds and ampicillin (reference antibacterial agent) were dissolved in DMSO in eppendorff tubes for final concentration of 5 mg/mL. Aliquots (100 µL) of each compound and ampicillin were applied into the wells, DMSO was also included as a negative control. The compounds were allowed to diffuse for 2 h at 4 °C and incubated at 37 °C for 24 h.\(^{16-18}\) Inhibition zones were measured using Vernier caliper and the activity of the tested compounds was estimated in comparison to ampicillin. The inhibition zone diameter (mm) of DMSO was subtracted from the antibacterial activity of the tested compounds.

**Determination of minimal inhibitory concentrations (MICs)**

Minimal inhibitory concentrations (MICs) were determined by serial dilution technique using 96-multiwell microtiter plates. The investigated compounds 2a, 2d, 3b, 3d and 3e were dissolved in DMSO 100% to prepare stock solutions of 5000 µg/mL. Two fold serial dilutions of the dissolved compounds were performed in LB broth providing eight different concentrations (2500, 1250, 625, 312.5, 156.25, 78.125, 39.06 and 19.53 µg/mL). Overnight
cultures of the tested bacteria were diluted to $1 \times 10^6$ CFU/mL in LB broth, and 20 µL of the diluted cultures were added to the test solutions (50 µL) in the wells (one well per concentration per bacterial strain). The plates were incubated at 37 °C for 24 h. MIC was detected visually as the least concentration of the compound that inhibits bacterial growth (no turbidity), and the activity of the tested compounds was estimated in comparison to ampicillin.

**Antifungal assay**

**Determination of diameter of inhibition zone (mm)**

Sabouraud’s medium (50 mL) was inoculated with 50 µL of $1 \times 10^6$ CFU/mL of 24 h culture of *C. albicans*. Glucose minimal medium (50 mL) was inoculated with 50 µL of $1 \times 10^3$ SFU/mL of *A. fumigatus*. Wells were made in agar using cork borer. The tested compounds were dissolved in DMSO in eppendorff tubes for final concentration 5 mg/mL and 100 µL of the test solution was applied into the wells. The standard antifungal drug (fluconazole) was also added at the same concentration to each plate. In addition, DMSO (control solvent) was added to each plate. Plates were incubated at 37 °C for 48 h. Antifungal activity of the tested compounds was determined by measuring the inhibition zone diameter. The inhibition zone diameter of DMSO was subtracted from the antifungal activity of the tested compounds.

**Determination of minimal inhibitory concentrations (MICs)**

Minimal inhibitory concentrations (MICs) were determined by the serial dilution technique using 96-mutiwell microtiter plates. The investigated compounds 3b and 3e were dissolved in DMSO 100% to prepare stock solutions of 5000 µg/mL. Two fold serial dilutions of the dissolved compounds were performed in glucose minimal medium providing eight different concentrations (2500, 1250, 625, 312.5, 156.25, 78.125, 39.06 and 19.53 µg/mL). *A. fumigatus* was diluted to $1 \times 10^3$ SFU/mL in glucose minimal medium, and *C. albicans* was diluted to $1 \times 10^6$ CFU/mL in Sabouraud’s medium. The diluted cultures (20 µL) were added to the test solutions (50 µL) in the wells (one well per concentration). The plates were incubated at 30 °C for 48 hrs for *A. fumigatus*, and at 37 °C for 48 hrs for *C. albicans*. MIC was detected visually as the least concentration of the compound that inhibits fungal growth (no turbidity), and the activity of the tested compounds was estimated in comparison to fluconazole.

**Antiquorum-sensing assay**

The culture was prepared by growing *C. violaceum* in LB broth and incubated for 16-18 h in an orbital incubator running at 28 °C and 150 rpm. The culture was then adjusted to 0.5 McFarland standard (Ca. $1 \times 10^6$ CFU/mL). *C. violaceum* (50 µL) was inoculated into LB agar (50 mL), poured into plates and solidified. Wells were made in LB agar medium using cork borer. The tested compounds 2a-f and 3a-e were dissolved in DMSO 100% in eppendorff
tubes for final concentration 5 mg/mL, and 50 µL of the test solution was applied into the wells. The positive control (indole) was also added at the same concentration and volume to each plate. In addition, DMSO (control solvent) was added to each plate. Plates were incubated at 30 °C for 48 h to check the inhibition of pigment production around the wells. Bacterial growth inhibition would result in a clear halo around the disc, while a positive quorum-sensing inhibition is exhibited by a turbid halo harboring pigmentless bacterial cells of C. violaceum ATCC 12472 monitor strain. Bacterial growth inhibition by the tested compounds was measured as radius ($r_1$) in mm, while both growth and pigment inhibition was measured as radius ($r_2$) in mm. The pigment inhibition (QS inhibition) was determined by subtracting bacterial growth inhibition ($r_1$) from the total radius ($r_2$); thus, QS inhibition = ($r_2$ - $r_1$) in mm.

**Antitumor evaluation**

*In vitro antiproliferative assay using three cancer cell lines*

**Materials**

The human cell lines were obtained from Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt.

**Procedure**

Compounds 2a-f and 3a-e were screened for *in vitro* antiproliferative activity against liver (HepG2), colon (HCT-116) and breast (MCF-7) cancer cell lines adopting MTT assay and using doxorubicin as a reference antiproliferative agent. Cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 µg/mL) at 37 °C in an atmosphere of 5% CO$_2$ were added.

Cells were placed in 96-multiwell microtiter plates (10$^4$ cells/well), for 24 h at 37 °C and in an atmosphere of 5% CO$_2$ before treatment with the compounds to allow attachment of the cells to the wall of the plate. The tested compounds were dissolved in DMSO and diluted with phosphate buffer saline (PBS) to obtain different concentrations. Tested compounds of different concentrations were added to each well and cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO$_2$. All tests were performed in triplicates. The treated cells were washed with PBS and 100 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (MTT) (5 mg/mL MTT stock in PBS diluted to 1 mg/mL with 10% RPMI-1640 medium) was added. The 96-multiwell plates were read by microarray reader PerkinElmer vector 3V multilabel counter model 1420 (PerkinElmer, Boston, MA) for optical density at 490 nm. The relative percentage cell viability was calculated from the following equation:

\[
\text{% Cell viability} = \frac{A_{\text{treated cells}} - A_{\text{blank}}}{A_{\text{untreated cells}} - A_{\text{blank}}} \times 100
\]
The relation between surviving fraction and drug concentration is plotted to get the survival curve for HepG2, HCT-116 and MCF-7 cancer cell lines. The concentration required for 50% inhibition of cell viability (IC\textsubscript{50}) was obtained for each compound from the curve fitting using Sigma plot10.

**In vivo antitumor assay using EAC cells**

**Materials**

Adult Swiss male albino mice (weighing 20-25 g) were obtained from the Department of Pharmacology, Faculty of Pharmacy, Mansoura University, Egypt. They were kept in microlon boxes at temperature 25 ± 2 °C with a regular 12 h light/dark cycle. In addition, food and water were available.

EAC cells (procured from Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt) were harvested and prepared, then their total number was counted.

**Procedure**

7 Groups of mice \((n = 5)\) were used, and the experiments were repeated three times.

Group 1: Negative control (no EAC cells) - received normal saline.
Group 2: Positive control (EAC cells) - received normal saline.
Group 3: EAC cells - treated intraperitoneally with compound 2f (100 mg/kg).
Group 4: EAC cells - treated intraperitoneally with compound 3a (100 mg/kg).
Group 5: EAC cells - treated intraperitoneally with compound 3b (100 mg/kg).
Group 6: EAC cells - treated intraperitoneally with compound 3c (100 mg/kg).
Group 7: EAC cells - treated intraperitoneally with doxorubicin (100 mg/kg).

\(2 \times 10^6\) EAC cells were injected intraperitoneally in each mouse (groups 2-7). After one day, compounds 2f, 3a-c and doxorubicin were administered to mice for nine days, then blood samples were withdrawn for assessment of hematological parameters.

**Determination of tumor volume**

The ascetic fluid was gathered from the peritoneal cavity and its volume was determined. It was then centrifuged and the packed tumor cell volume was measured.

**Determination of viable tumor cell count**

100 \(\mu\)L Sample of EAC cells (from three mice per group) was utilized and diluted twenty times with saline. Cells were stained with trypan blue, viable cells are not stained, while the dead ones are stained. The number of viable cells was calculated.
**In vitro cytotoxicity assay using two normal cell lines**

Cytotoxic activity of pyrazolopyridines 2f and 3a-c was evaluated adopting the same procedure described under *in vitro* antitumor testing.[22,23]

**Mechanistic study**

**Methyl green/DNA displacement assay**

Methyl green/DNA (20 mg, Sigma, St. Louis, MO, USA) was suspended in 100 mL of 0.05 M Tris-HCl buffer, pH 7.5, containing 7.5 mM MgSO₄ and stirred at 37 °C with a magnetic stirrer for 24 h. Unless otherwise indicated, samples to be tested were dissolved in ethanol in eppendorf tubes. Solvent was removed under vacuum and 200 µL of methyl green/DNA solution was added to each tube. The absorption maxima for methyl green/DNA complex is 642.5-645 nm. Samples were incubated in the dark at ambient temperature. After 24 h, the final absorbance of samples was determined. Readings were corrected for initial absorbance and normalized as a % of the untreated methyl green/DNA absorbance value.[25] Concentrations required for 50% decrease in the initial absorbance of methyl green/DNA solution (IC₅₀) were determined for compounds 2a, 2f, 3a-c and 3e.

**Topoisomerase IIβ assay**

**Materials**

Human DNA topoisomerase IIβ (TOP IIβ) ELISA kit (Cat No. MBS942146, MyBioSource) was used for the quantitative determination of human DNA TOP IIβ concentrations in serum, plasma, tissue homogenates and cell lysates.

**Samples:** Serum, Plasma, Tissue homogenates, Cell lysates.

**Standard:** The standard vial was centrifuged at 6000-10000 rpm for 30s, and the standard was reconstituted with 1.0 mL of sample diluent.

**Reagents and quantities**

Standard (Freeze dried), Biotin-antibody (100 x concentrate) 1 x 120 µL, HRP-avidin (100 x concentrate) 1 x 120 µL, Biotin-antibody diluent 1 x 15 mL, HRP-avidin diluent 1 x 15 mL, Sample diluent 1 x 50 mL, Wash buffer (25 x concentrate) 1 x 20 mL, 3,3',5,5'-Tetramethylbenzidine (TMB) substrate 1 x 10 mL, stop solution 1 x 10 mL.

**Procedure**

Topoisomerase IIβ (TOP IIβ) assay was performed according to the manufacturer’s instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for TOP IIβ has been pre-coated onto 96-multiwell microplate. Standard
and samples are pipetted into the wells and any TOP IIβ present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for TOP IIβ is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, the tested compounds 2a, 2f, 3a-c and 3e were added to the wells and color develops in proportion to the amount of TOP IIβ bound in the initial step. Stop solution (50 µL) was added to each well. Read at 450 nm within 5 min. The color development was stopped and the intensity of the color was measured. Readings were converted to % inhibition for each compound and then repeated at different concentrations to determine the concentration that produce 50% enzyme inhibition (IC$_{50}$, µM).[26]
Supplementary Figure S1. (A) 3D Pharmacophoric map of 2a; pharmacophore color coding is yellow for hydrophobic regions, red for hydrogen acceptors and green for hydrogen donors. (B) 2D Pharmacophoric map of 2a; H is hydrophobic center, AR is aryl, HBA is hydrogen bond acceptor and HBD is hydrogen bond donor. (C) The overlay of pharmacophoric map of 2a to pharmacophoric map of the binding of etoposide to TOP IIβ.
**Supplementary Table S1.** TPSA, Nrotb and calculated Lipinski’s rule for 2a-f and 3a-e

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<sup>a</sup> TPSA: Topological polar surface area.
<sup>b</sup> Nrotb: Number of rotatable bonds.
<sup>c</sup> miLogP: The parameter of lipophilicity.
<sup>d</sup> nOH-NH: Number of hydrogen bond donor sites.
<sup>e</sup> nO-N: Number of hydrogen bond acceptor sites.
**Supplementary Table S2. Carcinogenicity and drug score of 2a-f and 3a-e**

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- non-carcinogenic; + carcinogenic
References


[2] LeadIT version 2.3.2; BioSolveIT GmbH, Sankt Augustin, Germany, 2017, www.biosolveit.de/LeadIT.


- 13 -


Sample Information

Report Date: 01/08/2018 03:35:51
Sample ID: SS46
Customer Name: Dr. Samia Salah - Pharmacy - Mansoura
Data File: C:/GCMSSolution/Data/Project1/SS46.QGD
Org Data File: C:/GCMSSolution/Data/Project1/SS46.QGD
Method File: C:/GCMSSolution/Data/Project1/High Temperature Op
Org Method File: C:/GCMSSolution/Data/Project1/High Temperature Op
Report File: C:/GCMSSolution/System/Tune1\default.qgt
Tuning File: C:/GCMSSolution/System/Tune1\default.qgt
Modified by: Dr. Mai Younis

Method

Analytical Line 1
IonSourceTemp: 250.00 °C
[MS Table]
- Group 1 - Event 1 -
Start Time: 0.000 min
End Time: 10.000 min
ACQ Mode: Scan
Event Time: 0.50 sec
Scan Speed: 1000
Start m/z: 50
End m/z: 500
Electron Voltage: 70 eV
Ionization Mode: EI

Spectrum

Mass Table

Line:1  R.Time:2.3(Scan#:274)
MassPeaks:311
RawMode:Single 2.3(274) BasePeak:57(457585)
BG Mode:None  Group 1 - Event 1

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<th>Rel. Int.</th>
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Molecular Weight: 355.40
Mansoura University
Faculty of Science
Spectral Analyses Unit
Chemistry department
ThermoFisher Nicolet IS10, USA
spectral range : 4000-400 cm⁻¹
DI Analysis
Shimadzu Qp-2010 Plus

Sample Information
Sample Name: SS45
Sample ID:
Customer Name: Dr. Samia Salah - Pharmacy - Mansoura
Data File: C:\GCMSsolution\Data\Project1\SS45.QGD
Org Data File: C:\GCMSsolution\Data\Project1\SS45.QGD
Method File: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File:
Tuning File: C:\GCMSsolution\System\Tune1\_default.qgt
EndFileModified by: Dr. Mai Younis
Modified: 01/08/2018 03:32:25

Method
--- Analytical Line 1 ---
Ion Source Temp: 250.00 °C
[MS Table]
--- Group 1 - Event 1 ---
Start Time: 0.00 min
End Time: 10.00 min
ACQ Mode: Scan
Event Time: 0.50 sec
Scan Speed: 1000
Start m/z: 50.00
End m/z: 500.00

Electron Voltage: 70 eV
Ionization Mode: EI

Mol. Wt. 398.47

Mass Table
Line#: 1 R.Time: 3.4 (Scan#: 405)
MassPeaks: 275
Raw Mode: Single 3.4 (405) Base Peak: 105 (76037)
BG Mode: None Group 1 - Event 1

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Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information
Analyzed by: Dr. Mai Younis
Analyzed: 01/08/2018 04:11:12
Sample Name: SS49
Sample ID:
Customer Name: Dr. Samia Salah - Pharmacy - Mansoura
Data File: C:\GCMSSolution\Data\Project1\SS49.QGD
Org Data File: C:\GCMSSolution\Data\Project1\SS49.QGD
Method File: C:\GCMSSolution\Data\Project1\High Temperature Op
Org Method File: C:\GCMSSolution\Data\Project1\High Temperature Op
Report File: C:\GCMSSolution\System\Tune1\default.gqt
Tuning File: C:\GCMSSolution\System\Tune1\default.gqt
Modified by: Dr. Mai Younis
Modified: 01/08/2018 04:16:47

--- Analytical Line 1 ---
IonSourceTemp: 2500.00 °C
[MS Table]
--Group 1 - Event 1--
Start Time: 0:00min
End Time: 10:00min
ACQ Mode: Scan
Event Time: 0.50sec
Scan Speed: 1000
Start m/z: 50.00
End m/z: 500.00
Electron Voltage: 70 eV
Ionization Mode: EI

Mol. Wt. 400.40

Spectrum

Mass Table
Line#: 1 R.Time: 3.7 (Scan#: 447)
MassPeaks: 370
RawMode: Single 3.7 (447) BasePeak: 185 (720042)
BG Mode: None Group 1 - Event 1

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DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Sample Name: SS47
Sample ID:
Customer Name: Dr. Samia Salah - Pharmacy - Mansoura
Data File: C:\GCMSolution\DataProject1\SS47.QGD
Org Data File: C:\GCMSolution\DataProject1\SS47.QGD
Method File: C:\GCMSolution\DataProject1\High Temperature Op
Org Method File: C:\GCMSolution\DataProject1\High Temperature Op
Report File:
Tuning File: C:\GCMSolution\System\Tune1\_default.qgt
SEndIfISModified by: Dr. Mai Younis
Modified: 01/08/2018 03:52:55

Analyzed by: Dr. Mai Younis
Analyzed: 01/08/2018 03:46:45

Method

Analytical Line 1
IonSourceTemp: 250.00 °C
[MS Table]
--Group 1 - Event 1--
Start Time: 00.00 min
End Time: 10.00 min
ACQ Mode: Scan
Event Time: 0.50 sec
Scan Speed: 1000
Start m/z: 50.00
End m/z: 500.00
Electron Voltage: 70 eV
Ionization Mode: EI

Mol. Wt: 434.84

Spectrum

Mass Table
Line#: 1 R.Time: 1.8(Scan#: 211)
MassPeaks: 349
RawMode: Single 1.8(211) BasePeak: 57(189572)
BG Mode: None Group 1 - Event 1

# m/z Abs. In Rel. Int. # m/z Abs. In Rel. Int. # m/z Abs. In Rel. Int.
1 50.00 43908 23.16 4 53.00 22174 11.70 7 56.05 42049 22.18
2 51.00 31886 16.82 5 54.10 13308 7.02 8 57.05 189572 100.00
3 52.00 10498 5.54 6 55.05 124809 65.84 9 58.05 11249 5.93
Mon Jul 24 12:49:06 2017 (GMT+02:00)

Number of sample scans: 32
Number of background scans: 32
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.6329
Aperture: 80.00

Mansoura University
Faculty of Science
Spectral Analyses Unit
Chemistry department
ThermoFisher Nicolette IS10, USA
spectral range: 4000-400 cm⁻¹
Sample Information

Method

Analytical Line 1
IonSourceTemp : 250.00 °C
[MS Table]
--Group 1 - Event 1--
Start Time : 0.00min
End Time : 10.00min
ACQ Mode : Scan
Event Time : 0.50sec
Scan Speed : 1000
Start m/z : 50.00
End m/z : 500.00
Electron Voltage : 70 eV
Ionization Mode : EI

Mol. Wt. 355.41

Mass Table

Line#: 1 R.Time: 4.3(Scan#: 513)
MassPeaks: 311
RawMode: Single 4.3(513) BasePeak: 185 (168302)
BG Mode: None Group 1 - Event 1

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Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information
Analyzed by: Dr. Mai Younis
Analyzed: 01/08/2018 02:33:56
Sample Name: SS40
Sample ID:
Customer Name: Dr. Samia Salah - Pharmacy - Mansoura
Data File: C:\GCMSsolution\Data\Project1\SS40.QGD
Org Data File: C:\GCMSsolution\Data\Project1\SS40.QGD
Method File: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File:
Tuning File: C:\GCMSsolution\System\Tune1\_default.qgt
$EndIf$ Modified by: Dr. Mai Younis
Modified: 01/08/2018 02:42:11

C:\GCMSsolution\Data\Project1\SS40.QGD

Method
Analytical Line 1
IonSourceTemp: 250.00 °C
[MS Table]
Group 1 - Event 1
Start Time: 0:00.00min
End Time: 1:00.00min
ACQ Mode: Scan
Event Time: 0.50sec
Scan Speed: 1000
Start m/z: 50.00
End m/z: 500.00

Electron Voltage: 70 eV
Ionization Mode: EI

Mol. Wt. 389.85

Spectrum

Mass Table
Line#: 1 R.Time: 2.1 (Scan#: 254)
MassPeaks: 341
RawMode: Single 2.1 (254) BasePeak: 57(670581)
BG Mode: None Group 1 - Event 1

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Mon Jul 24 13:03:21 2017 (GMT+02:00)

Number of sample scans: 32
Number of background scans: 32
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.6329
Aperture: 80.00

**Thermo Fisher Scientific**

Mansoura University
Faculty of Science
Spectral Analyses Unit
Chemistry department
ThermoFisher Nicolet IS10, USA
spectral range: 4000-400 cm⁻¹
Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Sample Name: SS41
Sample ID: 
Customer Name: Dr. Samia Salah - Pharmacy - Mansoura
Data File: C:\GCMSSolution\Data\Project1\SS41.QGD
Org Data File: C:\GCMSSolution\Data\Project1\SS41.QGD
Method File: C:\GCMSSolution\Data\Project1\High Temperature Op
Org Method File: C:\GCMSSolution\Data\Project1\High Temperature Op
Report File: 
Tuning File: C:\GCMSSolution\System\Tun1\default.qgt
Send/Receive/Modified by: Dr. Mai Younis
Modified: 01/08/2018 02:47:24

Method

Analytical Line 1
IonSourceTemp: 250.00 °C
[MS Table]
--Group 1 - Event 1--
Start Time: 0.00min
End Time: 10.00min
ACQ Mode: Scan
Event Time: 0.50sec
Scan Speed: 1000
Start m/z: 50.00
End m/z: 500.00

Electron Voltage: 70 eV
Ionization Mode: EI

Spectrum

3c
Mol. Wt. 398.47

Mass Table
Line#: 1 R.Time: 3.9(Scan#: 473)
MassPeaks: 304
RawMode: Single 3.9(473) BasePeak: 57(51041)
BG Mode: None Group 1 - Event 1
Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Sample Name: SS34
Sample ID: 07/08/2018 10:11:50

Customer Name: Dr. Samia Salah - Pharmacy - Mansoura

Data File: C:\GCMSolution\Data\Project1\SS34.QGD
Method File: C:\GCMSolution\Data\Project1\SS34.QGD
Org Data File: C:\GCMSolution\Data\Project1\High Temperature Op
Org Method File: C:\GCMSolution\Data\Project1\High Temperature Op
Report File: C:\GCMSolution\System\Tune1\default.qgt
Mod Date/Modified by: Dr. Mai Younis

Spectrum

Mol. Wt. 480.59

Mass Table

Line#: 1 R.Time: 2.8(Scan#: 336)
MassPeaks: 334
RawMode: Single 2.8(336) BasePeak: 269(121904)
BG Mode: None Group 1 - Event 1

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