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G. Lakshma Reddy, Santosh Kumar Guru, M. Srinivas, Anup Singh Pathania, Priya Mahajan, Amit Nargotra, Shashi Bhushan, Ram A. Vishwakarma, Sanghapal D. Sawant

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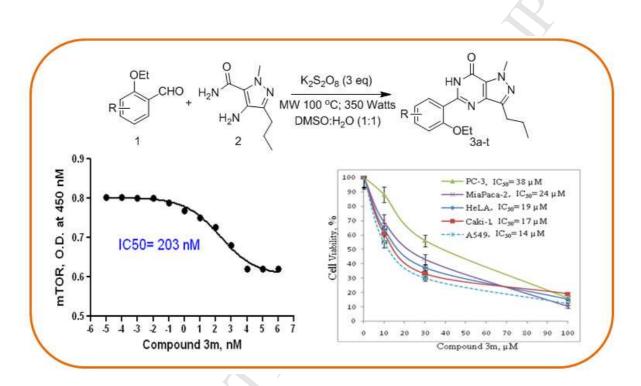
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Graphical Abstract

Synthesis of 5-substituted-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one analogs and their biological evaluation as anticancer agents: mTOR inhibitors

G. Lakshma Santosh Kumar Guru, Reddy, M. Srinivas, Anup Singh Pathania, Priya Mahajan, Amit Nargotra, Shashi Bhushan,* Ram A. Vishwakarma,* Sanghapal D. Sawant*



Series of 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6H)-ones are synthesized using microwave assisted strategy. Screened against HeLa, CAKI-I, PC-3, MiaPaca-2, A549 for anticancer activity and 3m was found to be an mTOR inhibitor.

Synthesis of 5-substituted-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one analogs and their biological evaluation as anticancer agents: mTOR inhibitors

G. Lakshma Reddy,^{a,d} Santosh Kumar Guru,^b M. Srinivas,^{a,d} Anup Singh Pathania,^{b,d} Priya Mahajan,^{c,d} Amit Nargotra,^{c,d} Shashi Bhushan,^{b,d}* Ram A. Vishwakarma,^{a,d,*} Sanghapal D. Sawant,^{a,d,*}

^aMedicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Canal Road-Jammu-180 001, India ^bCancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road-Jammu-180 001, India ^cDiscovery Informatics, CSIR-Indian Institute of Integrative Medicine, Canal Road-Jammu-180 001, India ^dAcademy of Scientific and Innovative Research, India Tel.: +91-191-2569111, Fax: +91-191-2569333; IIIM Communication No. IIIM/1610/2013 E-mail: sdsawant@iiim.ac.in, sawant.rrl@gmail.com

Abstract:

A microwave assisted strategy for synthesis of series of 1H-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)ones has been developed and their biological evaluation as anticancer agents is described. The synthetic protocol involves simple procedure by oxidative coupling of 4-amino-1-methyl-3propyl-1*H*-pyrazole-5-carboxamide with different aldehydes in presence of K₂S₂O₈ offering 5substituted-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one compounds in excellent yields. The *in vitro* anticancer activity screening against human cancer cell lines HeLa, CAKI-I, PC-3, MiaPaca-2, A549 gave good results. The in detailed mechanistic correlation studies of compound **3m** revealed that the compound shows anticancer activity through apoptosis mechanism and also inhibits mTOR with nonomolar potency. The design was based on docking with mTOR protein. The concentration dependent cell cycle analysis, western blotting experiment and nuclear cell morphology studies have been described.

Keywords: Pyrazolo[4,3-d]pyrimidin-7(6H)-one, K₂S₂O₈ catalyst, Microwave irradiation, Cytotoxicity, mTOR inhibitor

1.0. Introduction

The pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-ones and their bioisosteres are heterocyclic compounds with important biological functions including antitumor activity and many other activities.¹ 6-cycloalkyl-pyrazolopyrimidinones are reported for CNS disorders,² GHS-R1a antagonists and inverse agonists for the treatment of obesity is also repoted.³ Recently, imine-pyrazolopyrimidinones are presented as anticancer derivatives.⁴ 1-aryl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones were identified as inhibitors of cyclin-dependent kinase (CDK) with IC₅₀ in the low micromolar range⁵ and several other reports are available for various activities for this scaffold. On the basis of biological data reported in literature, pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one class of compounds are very important in the treatment of impotence, as can be evidenced by the top selling PDE5 inhibitory drug in market *i.e.*, sildenafil.⁶ Few more to include in pyrazolopyrimidinone class other than sildenafil as PDE5 inhibitors are acetildenafil (hongdenafil), aildenafil (methisosildenafil), sulfoaildenafil (thioaildenafil), etc.

A pyrazolopyrimidine scaffold based molecule *i.e.* PP242, PP30 and some others⁷ (Fig. 1), are reported as highly potent, selective and ATP-competitive mTORC1/mTORC2 inhibitor (IC₅₀ = 8 nM for PP242 and 80 nM for PP30). PP242 has >10 folds selectivity over the other PI3K family kinases (IC₅₀ 0.102 μM, 0.408 μM, 1.27 μM, 1.96 μM and 2.2 μM for p110γ, DNA-PK, p110δ, p110 α and p110 β , respectively). PP242 is also reported to exhibits excellent selectivity over 215 other protein kinases. PP242 differentially inhibits insulin-stimulated phosphorylations of cellular proteins both in vitro and in vivo in a manner distinctly different from that seen in mTORC2-functional knockout SIN1^{-/-} cells or in cultures treated with Rapamycin, which targets only mTORC1, but not mTORC2. Moreover, it is reported that PP242 can significantly enhance iPSC generation, which is experimentally confirmed by J.A Menendez et. al. that this mTOR inhibitor (PP242) is the most powerful longevity-promoting molecule that enhances iPSC generation,⁸ and robustly decelerates the cellular senescence imposed by a DDR equivalent to senescence that is caused by pluripotency associated transcription factor expression. However, support for this hypothesis was evidenced by recent findings that well-characterized mTOR inhibitors and autophagy activators (e.g., PP242, rapamycin and resveratrol) notably improve the speed and efficiency of iPSC generation.⁸

Synthesis of pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one is well exploited and there are various methods already reported for the synthesis of pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one class of compounds using traditional as well as microwave mediated approaches.⁹ The aim of this study was to synthesize and evaluate the biological potential of pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one analogs for their anticancer potential. In this direction, we initiated our efforts towards its synthesis and biological activity. The detailed chemistry and biological evaluation of these compounds as anticancer agents is explained in the present study.

2.0. Result and discussion

2.1. Chemistry:

2.1.1. Synthesis of 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one analogs:

We intended to synthesize compounds based on 1H-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one scaffold by using microwave assisted protocol (Scheme 1). In this direction we started the studies for optimization of synthesis of 5-(2-ethoxyphenyl)-1-methyl-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6H)-one **3a**.

The optimization studies were initiated by screening of different oxidizing agents as depicted in table 1 using DMSO:Water in 1:1 proportion to see the conversion in desired product. Amongst all oxidants, the best result was observed with $K_2S_2O_8$, in equivalence studies for catalyst, 3 eq. of catalyst has given maximum yields (Table 1, see Supplementary data). Therefore, all reactions were conducted using this condition after optimization of catalyst. However, oxone has also given the product **3a** with minor yields.

After screening of the catalyst we started study of selectivity for solvent that could affect the formation of 5-(2-ethoxyphenyl)-1-methyl-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-7(6*H*)-one **3a**. The solvent screening was carried out to find out the best conversion, the mixture of DMSO:H₂O in 1:1 proportion has given the best results with excellent yields (Table 2, see Supplementary data).

The microwave protocols were optimized for this reaction as mentioned in the table 3; the reactions carried under different microwave Watt powers have given varied results. Wherein, entry 3(b) (Table 3, see Supplementary data) was found to be the best condition for maximum conversion.

A series of compounds based on 1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one scaffold was synthesized using these optimized conditions, wherein, all kind of substrates with diversity around aryl ring were chosen for conversion and in all cases products obtained in good to excellent yields (Table 4).

2.2. Biology:

2.2.1. In vitro anticancer activity

These compounds were taken up for *in vitro* cell based cytotoxicity screening against various human cancer cell lines and the results for this screening are mentioned in the Table 5.

2.2.2. Compound **3m** inhibits significant cell growth inhibition in different panel of human cancer cell lines:

Cytotoxicity assay was performed by using tetrazolium based calorimetric method (MTT assay) against human lung cancer cell lines A549, kidney cancer cell line Caki-1, pancreateic cancer cell line MiaPaCa-2, prostate cancer cell line PC-3 and cervical cancer cell line HeLa. Compound **3m** caused concentration dependent inhibition of cell proliferation in these cell lines in 48 h (Fig.2). Compound **3m** has IC₅₀ value of approximately 14 μ M in A549 cell line, 17 μ M in Caki-1 cells, 24 μ M in MiaPaCa-2 cells, 38 μ M in PC-3 cells, in 19 μ M HeLa cells after 48 h.

2.2.3. Compound **3m** altered whole cell and nuclear morphology

Treatment of human leukaemia HL-60 cells with compound **3m** at 10, 30, 50, and 70μ M concentrations caused cell wall deformation, shrinkage of cell size, nuclear condensation and formation of scattered apoptotic bodies as shown by arrows in the Fig. 3A and B, while the nuclei of untreated cells are healthy and round in shape. The number of apoptotic bodies increased with increased concentration of compound **3m** in A549 cells. This revealed that compound **3m** induce cell death through induction of apoptosis in A549 cells.

2.2.4. Compound **3m** increases sub-G0 DNA fraction of cell cycle phase in concentration dependent manner

A549 cells treated with compound **3m** exhibited concentration dependent increase in hypo diploid sub-G0 DNA fraction (<2nDNA) indicative of apoptotic population as analyzed by modfit software (Fig. 4). Control cells showed 1% sub-G0 DNA fraction while A549 cells treated with compound **3m** for 24 h exhibited continuous increase in sub-G0 fraction which may comprise both apoptotic and debris fraction implying together the extent of cell death.

The damage was more apparent with higher concentration of compound **3m** over the period of study. The sub-G0 fraction increased from ~1% of control to ~28% after 24 h of treatment of compound **3m**. There was hardly any significant effect after 24h of treatment on G1, S and G2/M, which indicated that decrease in DNA fluorescence is not cell cycle selective.

2.2.5. Compound **3m** inhibits mTOR-p70S6Kinase signaling and induces apoptosis in A549 cells

Compound **3m** at indicated concentrations inhibits phosphorylated (Serine 2448) and non phosphorylated form of mammalian target of rapamycin (mTOR), its two other subunits rictor and raptor and their two main substrate p70S6K and 4EBP1in A549 cells after 24h (Fig.5). Compound **3m** inhibits active phosphorylated form of eIF4E (Serine 209) and p-p70S6Kinase (T389) at all concentrations (Fig.5).

Compound **3m** also inhibits mTOR kinase in a cell free enzyme assay (K-LISATM mTOR kit) and IC₅₀ value was found to be 203 nM (Fig. 6). The mammalian target of rapamycin (mTOR) is a serine/threonine kinase present downstream of phosphatidylinositol 3-kinase/Akt signalling pathway and it involves in regulating basic cellular functions including cellular growth and proliferation (Wullschleger etal, 2006).¹⁰ Aberrant activation of the PI3K/Akt/mTOR pathway is found in many types of cancer including small cell lung cancer (Marinov etal. 2009).¹¹ AKT can activate mTOR by phosphorylating at serine 2448. Activated mTOR have two well characterized downstream targets, ribosomal protein S6 kinase 1 (p70S6K) and eukaryotic translation initiation factor 4E binding protein 1 (4EBP1), both of which are involve in the regulation of protein synthesis (Hay and Sonenberg, 2004).¹² Simultaneously, compound **3m** also alter the expression of key apoptotic proteins like caspas-3 activation and PARP-1 (Poly-ADP-ribose-polymerase) cleavage (Fig. 5).

2.3. Docking studies

Based on the docking studies, the molecule 3m shows one H-bond interaction with Val2240 at a distance of 2.203 A⁰ (Fig. 7). Further, this ligand also shows more stability within the binding pocket due to the hydrophobic cleft formed by Leu 2185, Trp 2239, Met 2345 and Ile 2356 around the ligand. The best dock score and the calculated binding energy of this complex were - 6.8 and -80.95 respectively. Based on this evidence and the preliminary biological activity, the in detailed biological activity for 3m was conducted.

3.0. Conclusion

In present study, 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one scaffold have been constructed using microwave assisted protocol and the biological evaluation results as anticancer agents are promising. The synthetic protocol can be applied for preparation of analogs of active compound i.e. **3m** which involves a simple procedure to obtain 5-substituted-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one compounds in excellent yields. Moreover, **3m** also acts as mTOR inhibitor and the concentration dependent cell cycle analysis, western blotting experiment and nuclear cell morphology studies suggests that the mechanism through which 3m acts as anticancer agent is apoptosis. 5-substituted-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one is an excellent scaffold and can be exploited for further study in the field of cancer.

4.0. Experimental protocols

4.1.1. Chemistry

All reactions were performed in a sealed tube under mentioned microwave irradiation conditions. Analytical thin layer chromatography was performed using TLC pre-coated silica gel 60 F_{254} (20 x 20 cm). TLC plates were visualized by exposing UV light or by iodine vapors or immersion in anisaldehyde charring reagent or in 2,4-dinitrophenyl hydrazine or ninhydrin followed by heating on hot plate. Organic solvent were concentrated by rotary evaporation and dried using high vacuum suction pump. Compounds were purified by column chromatography using normal phase silica-gel (100-200 mesh size). ¹H NMR spectra were recorded with 400 and 500 MHz NMR instruments. Chemical data for protons are reported in parts per million (ppm, scale) downfield from tetramethylsilane and are referenced to the residual proton in the NMR solvent (CDCl₃: δ 7.26, DMSO-d₆ δ 2.50 or other solvents as mentioned).

4.1.1.1. Synthesis of 1-methyl-3-propyl-5-(2,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3d]pyrimidin-7(6H)-one

In a typical procedure, a solution of aromatic aldehyde i.e. 2,4,5-trimethoxybenzaldehyde (0.196 g, 1 eq.) and 4-amino-1-methyl-3-propyl-1*H*-pyrazole-5-carboxamide (0.191 g, 1.05 eq) in DMSO:H₂O (1:1) add $K_2S_2O_8$ (0.810 g, 3 eq.) was taken in a sealed reaction tube and the reaction mixture was irradiated under microwave conditions for 3 min with a power of 350 Watts at 100 °C. After completion, the reaction mass was diluted with EtOAc (20 mL) and added water (30 mL). Separated the organic layer and extracted with EtOAc (2x10ml). The combined

organic layer was then washed with brine solution, concentrated under vacuum and purified on silica-gel (100-200 mesh) column chromatography, affording white solid 1-methyl-3-propyl-5-(2,4,5-trimethoxyphenyl)-1*H*-pyrazolo[4,3-d]pyrimidin-7(6H)-one i.e., compound **3j** in (0.351 g) 98% yield.

4.1.1.2. Representative analytical data for compound **3***j* i.e., 1-methyl-3-propyl-5-(2,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one. ¹H NMR (400 MHz, CDCl₃) δ 10.99 (s, 1H), 8.03 (s, 1H), 6.59 (s, 1H), 4.27 (s, 3H), 4.05 (s, 3H), 3.97 (s, 6H), 2.93 (t, *J* = 7.5 Hz, 2H), 1.93 – 1.83 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 153.61, 152.09, 151.83, 149.06, 144.66, 142.60, 137.96, 123.88, 113.50, 112.81, 97.89, 56.57, 56.15, 55.89, 37.77, 27.07, 21.59, 13.81. HRMS (ESI) calcd for C₁₈H₂₃N₄O₄ [M-H⁺] 359.17193, found 359.17139.

4.1.2. Molecular modeling studies

The computational studies on mTOR were carried out using the Schrodinger suite 2012 molecular modeling software. Crystal structure 4JT5 was taken for docking studies. The coordinates of mTOR protein in complex with the co-crystalized ligand (2-[4-amino-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]-1H-indol-5-ol) that was obtained from protein data bank.¹³ The protein was prepared for docking using the protein preparation wizard. Hydrogens were added to the protein and water residues were removed beyond 5A° from the heteroatom. Further, only those water residues, having interactions with the protein and heteroatom were kept, and the rest were deleted. Then the ligand was extracted and protein was refined by assigning H-bonds and minimization at OPLS 2005 force field. A grid was generated at active site, identified on the bases of already co-crystalised ligand to the receptor using receptor grid generation module. The docking protocol was standardized using the co-crystalized ligand conformation.

4.1.3. Biological Studies

a) Reagents/chemicals: RPMI-1640, streptomycin, kanamycin, penicillin, L-glutamine, phenylmethanesulfonyl fluoride (PMSF), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Propidium iodide (PI), Fetal bovine serum, pyruvic acid were purchased from Sigma-Aldrich (Bangalore, India). Anti-human antibodies to PARP-1, caspase-3 and β -actin were purchased from SantaCruz Biotechnology (SantaCruz, CA). Anti-human antibodies to

mTOR and its phosphorylated form (S2448), p-eIF4E (S209), Raptor, Rictor and p-p70S6Kinase (T389) were purchased from Cell signaling technology (Danvers, MA). K-LISA[™] mTOR kit (#CBA055) was purchased from Calbiochem, USA. Electrophoresis reagents, Protein estimation kit and protein marker were from Bio-Rad Laboratories (Hercules, CA).

b) Cell culture and treatment: Human lung cancer cell lines A549, kidney cancer cell line Caki-1, pancreatic cancer cell line MIA-PaCa-2, prostate cancer cell line PC3 and cervical cancer cell line HeLa were purchased from ECACC. Cells were grown in RPMI/DMEM/MEM growth medium containing 10% FCS, 100U penicillin and 100 mg/ml streptomycin. Cells were grown in CO_2 incubator (Thermocon Electron Corporation, Houston, TX) at 37 °C temperature, 95% humidity and 5% CO_2 gas environment. Cells treated with compounds were dissolved in DMSO while the untreated control cultures received only the vehicle (DMSO<0.2%).

4.1.3.1. Cell proliferation assay

Cells were seeded in 96 well flat bottom plates. Next day when they attained 60-70% confluency, they were treated with compounds at different concentrations for 24h and 48h. MTT dye (2.5 mg/ml in PBS) was added 4 hours priors to experiment termination. The supernatant was discarded and the MTT formazan crystals were dissolved in 150 μ L of DMSO. The OD measured at 570 nm with reference wavelength of 620 nm (Bhushan et al 2006).¹⁴ The most active compound **3m** was taken up for in detailed biological evaluation.

4.1.3.2. Hoechst Staining

Human lung cancer A549 cells were treated with compound **3m** for 24 h at 10, 30, 50 and 70 μ M concentrations. After treatment cells were collected, washed twice with PBS and fixed in 400 μ l of fixing solution composed of cold acetic acid: methanol (1+3, v/v) overnight at 4 °C. Cells were washed with fixing solution and dispensed in 50 μ l of fixing solution. Spread cells on a clean cold slide and dried overnight at room temperature. Cells were stained with Hoechst 33258 (5 μ g/ml in 0.01 M citric acid and 0.45 M disodium phosphate containing 0.05% Tween 20) for 20-30 min at room temperature. After that slides were washed with distilled water followed by in PBS. While wet, pour 40 μ l of mounting fluid (PBS: glycerol, 1/1) over the slide and covered with glass cover slip and sealed with nail polished. Cells were observed under microscope for any nuclear morphological changes occur in apoptosis (Saxena et al. 2010).¹⁵

4.1.3.3. Cell cycle analysis

Human lung cancer cell line A549 was seeded in 60mm^2 dishes and after attaining 60-70% confluency, treated with compound **3m** for 24 hours at 10, 30, 50 and 70 µM concentrations. Cells were washed with PBS twice after 24h and fixed overnight in 70% alcohol. Next day cells were washed twice with PBS and thereafter they are subjected to RNase digestion (200 µg/mL) at 37^{0} C for 1.30 h and then incubated with PI (10 µg/mL) for 30 min. Cells were analyzed immediately on flow cytometer (BD Biosciences, San Jose, CA). The data were collected in list mode on 10,000 events and illustrated in a histogram, where the number of cells (counts) is plotted against the relative fluorescence intensity of PI (FL-2; λ em: 585 nm; red fluorescence). Resulting DNA distributions were analyzed by Modfit (Verity Software House Inc., Topsham, ME) for the proportions of cells in G₀-G₁, S- phase, and G₂-M phases of the cell cycle (Chanda et al 2012).¹⁶

4.1.3.4. Cell lysates preparation for western blots analysis

Human lung cancer cell line A549 was treated with compound **3m** for 24h at 10, 30, 50 and 70 μ M concentrations. Cells were collected by centrifugation at 400×g at 4^oC, washed with PBS twice and processed for preparation of whole cell lysates. Cells were lysed with cold Cell lysis buffer (RIPA, Sigma with 50 mM NaF, 0.5 mM NaVO₄, 2 mM PMSF and 1% protease inhibitor cocktail) for 40 min. Cells were centrifuged at 12000× g for 15 min at 4^oC and the supernatant was collected as whole cell lysates for western blot analysis (Bhushan et al 2007).¹⁷

4.1.3.5. Western blot analysis

Protein was measured using Bio-Rad protein assay kit and protein lysates (30-70 μ g) were subjected to SDS-PAGE analysis. They are transferred to PVDF membrane for 2 hours at 4^oC at 100V using Bio-Rad electrode assembly. The membrane was blocked by incubation with 3% BSA or 5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature to prevent any non-specific binding. After an hour the blots were incubated with respective primary antibodies for 3-4 h at room temperature. They are washed three times with TBST and were incubated with horseradish peroxidase conjugated secondary antibodies for another 1 h. At the end, membranes were washed three times with TBST buffer with 15 min interval and signals detected using ECL plus chemiluminescence's kit on X-ray film (Bhushan et al 2007).¹⁷

4.1.3.6. mTOR kinase essay

mTOR inhibition of compound **3m** was found out by using K-LISATM mTOR kit from Calbiochem (#CBA055). It is an ELISA-based assay that utilizes a p70S6K-GST fusion protein as a specific mTOR substrate. The assay was carried out according to the manufacturer's protocol. Briefly, 100 µl of recombinant p70S6K-GST fusion protein was pre-incubated at room temperature in the glutathione coated 96-well plate for 1h after that a mixture of 49µl of ice-chilled mTOR kinase and 1µl of test compounds or DMSO was added. The reaction was initiated by the addition of 50 µl of mTOR kinase assay buffer containing 100 µM ATP and 1µM DTT. The plate was treated first with 100 µl of anti-p70S6K-T389 for 1h and then with 100 µl of HRP-conjugated antibody for 1h to detect the T389-phosphorylated p70S6K. Absorbance was measured at 450 nm and 595 nm using microplate spectrophotometer. The IC₅₀ values were calculated by analysis non linear regression with variable slope by using GraphPad Prism-5 software.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://

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Figure captions

Figure 1: Pyrazolo[4,3-d]pyrimidin-7(6H)-one scaffold based potential candidates and drugs

Figure 2: Compound 3m inhibits cell proliferation in different panel of human cancer cell lines. Cells were grown in 96-well culture plate and when 60-70% confluent was treated with 1, 10, 30, 50, 70 and 100 μ M concentration for 48h. Cells were incubated with MTT and OD measured as described in Materials and Methods. Data are Mean ± SD (n= 8 wells), and representative of three similar experiments **Figure 3:** Effect of compound 3m on the cell wall and nuclear morphology of A549 cells. A) A549 cells were treated with 10, 30, 50 and 70 μ M concentrations of compound 3m for 24h and visualized under phase contrast inverted microscope (Olympus 1X 70, 30X). B) Subsequently cells were stained with Hoechst 33258 and visualized for nuclear morphology and apoptotic bodies' formation. Compound 3m induced the formation of apoptotic bodies as indicated by arrows in concentration dependent manner. Data are representative of one of three similar experiments

Figure 4: Cell cycle analyses of lung cancer A549 cells through PI staining. Cells were seeded in six well plate and after 60-70% confluence, treated with compounds 3m for 24h time period at indicated concentrations. After treatment cells were stained with PI ($10\mu g/ml$) to determine DNA Fluorescence and cell cycle phase distribution as described in Materials and methods. Data were analyzed by Modfit software (Verity Software House Inc., Topsham, ME) for the proportions of different cell cycle phases. Fraction of cells from apoptotic, G1, S and G2 phases analyzed from FL2- A vs. cell counts is shown in %. Data are representative of one of three similar experiments.

Figure 5: Inhibition of mTOR signalling cascade in lung cancer A549 cells by compounds 3m. A549 cells treated with compound 3m at 10, 30, 50 and 70 μ M concentrations for 24 h. Total cell lysates were prepared and protein samples (40-80 μ g) were loaded on SDS-PAGE gel for western blot analysis, β -actin was used as internal control to represent the same amount of proteins applied for SDS-PAGE. Specific antibodies were used for detection of mTOR, its activated phosphorylated form at serine 2448, Raptor, Rictor along with downstream substrates of mTOR, eIF4E and p70S6kinase. Compound 3m also induces apoptotic caspase-3 level and PARP cleavage. Immunoblots were representative of one of three similar experiments.

Figure 6: Compound 3m inhibits mTOR kinase in a cell free enzyme essay (K-LISATM mTOR kit) showing IC_{50} value as 203 nM.

Figure 7: A cartoon showing the interactions of compound 3m with active site of mTOR using PDB4JT5 (The carbonyl group of molecule showing H-bonding with Val2240 (2.203 A^0))

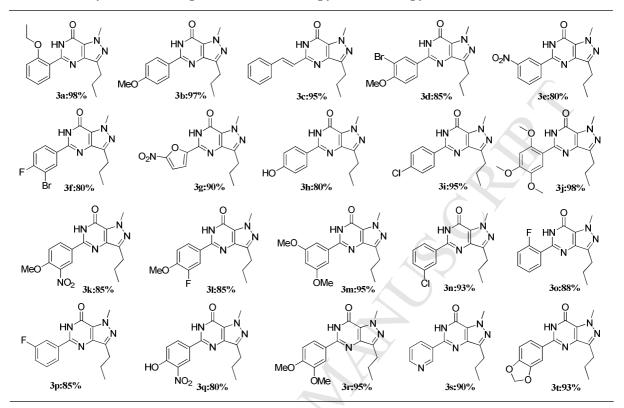


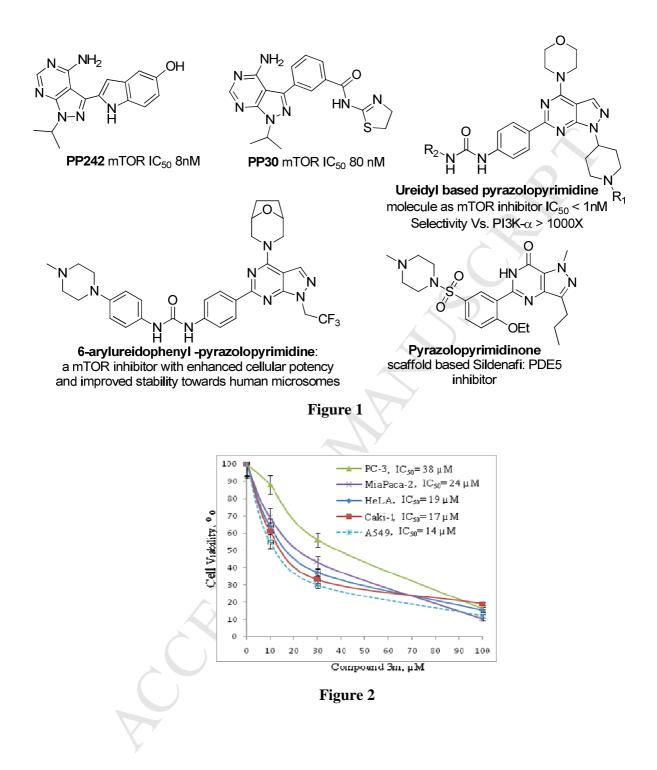
Table 4: Synthesis of compounds based on 1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one scaffold

^aAll yields are isolated yields after column purification

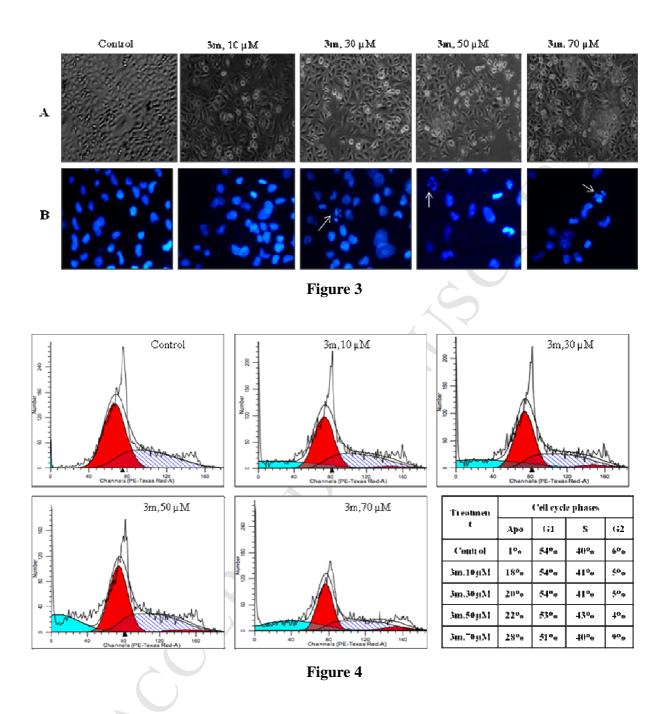
Table 5: In vitro cell based screening of 1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one compounds

							C	ell lin	es						
		HeLa		0	AKI	·I		PC-3		Mi	aPaca	a-2		A549	
Code	(Cerv	vix cai	ncer)	(Ren	al ca	ncer)	(Prost	ate Ca	ancer)	(Pa	ncrea	ntic	(Luı	ng car	ncer)
										C	ancer	;)			
	Conc,	%	IC_{50}	Conc,	%	IC ₅₀	Conc,	%	IC_{50}	Conc,	%	IC_{50}	Conc,	%	IC ₅₀
	(µM)	GI	(µM)	(µM)	GI	(µM)	(µM)	GI	(µM)	(µM)	GI	(µM)	(µM)	GI	(µM)
	10	36		10	12		10	7		10	7		10	21	
3a	30	37	>100	30	18	>100	30	10	>100	30	25	>100	30	30	>100
	100	40		100	19		100	14		100	38		100	37	
	10	31		10	8		10	5		10	18		10	0	>100
3b	30	34	>100	30	25	>100	30	6	>100	30	20	>100	30	15	
	100	42		100	52		100	14		100	32		100	36	
	10	22		10	26		10	33		10	11		10	30	38
3c	30	43	92	30	33	>100	30	48	32	30	20	>100	30	45	
	100	59	7	100	46		100	98		100	44		100	80	
	10	0		10	24		10	14		10	11		10	0	90
3d	30	21	>100	30	38	57	30	16	>100	30	15	>100	30	17	
	100	36		100	74		100	19		100	22		100	58	
	10	21		10	31		10	22		10	14		10	12	>100
3e	30	22	>100	30	50	30	30	27	>100	30	17	>100	30	25	
	100	39		100	73		100	47		100	45		100	40	

	10	34		10	30		10	32		10	34		10	20	28
3f	30	69	24	30	68	18	30	62	27	30	45	38	30	57	
	100	81		100	78		100	74		100	98		100	98	
	10	12		10	27		10	10		10	6		10	0	>100
3g	30	17	>100	30	32	>100	30	23	>100	30	16	>100	30	8	
U	100	48		100	41		100	39		100	32		100	43	
	10	29		10	22		10	19		10	23		10	11	90
3h	30	44	34	30	25	>100	30	24	>100	30	29	>100	30	28	10
011	100	78	0.	100	35	/ 100	100	32	, 100	100	43	. 100	100	59	
	100	12		100	34		100	22		100	11		100	19	87
3i	30	19	>100	30	56	28	30	31	>100	30	22	>100	30	39	07
51	100	19	>100	100	66	20	100	47	>100	100	43	>100	100	58	
				100						100	12		100		> 100
2;	<u>10</u> 30	<u>30</u> 33	>100	30	16	>100	<u>10</u> 30	<u>5</u> 7	>100	30		>100		0	>100
3ј			>100		16	>100			>100	11	21	>100	30	7	
	100	35		100	17		100	10		100	32	/	7	21	. 100
21	10	32	. 100	10	25	. 100	10	0	. 100	10	3	. 100	10	11	>100
3k	30	35	>100	30	31	>100	30	6	>100	30	24	>100	30	31	
	100	43		100	35		100	13		100	44		100	47	
	10	49		10	35		10	33		10	38		10	26	28
31	30	66	13	30	65	20	30	78	31	30	57	35	30	57	
	100	87		100	87		100	97		100	87		100	90	
	10	36		10	39		10	12		10	31		10	45	14
3m	30	63	19	30	67	17	30	44	37	30	57	24	30	68	
	100	85		100	81		100	84		100	90		100	98	
	10	22		10	35		10	16		10	12		10	0	87
3n	30	31	>100	30	51	29	30	17	>100	30	16	>100	30	27	
	100	49		100	76		100	25		100	23		100	65	
	10	11		10	24		10	17		10	11		10	0	>100
30	30	43	35	30	26	>100	30	23	>100	30	20	>100	30	8	
	100	65		100	49		100	43		100	43		100	23	
3р	10	1	>100	10	34	96	10	23	95	10	15	>100	10	22	86
-	30	18		30	47		30	44		30	21		30	37	
	100	33		100	54		100	56		100	44		100	68	
	10	0		10	23		10	21		10	17		10	0	>100
3q	30	7	>100	30	35	>100	30	25	>100	30	22	>100	30	23	
1	100	12		100	44		100	29		100	48		100	46	
	10	13		10	21		10	11		10	21		10	9	>100
3r	30	21	>100	30	28	>100	30	14	>100	30	32	96	30	14	
	100	44		100	36		100	27		100	55		100	48	
	100	0	(10	21		100	1		100	21		100	0	>100
3s	30	23	>100	30	22	>100	30	3	>100	30	32	>100	30	10	00
20	100	38	. 100	100	31	- 100	100	14	- 100	100	45	- 100	100	29	
	100	32)	100	46		100	23		100	11		100	10	54
3t	30	44	88	30	55	27	30	31	98	30	13	>100	30	37	57
JL	100	61	00	100	61	21	100	51	20	100	34	/100	100	78	



ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

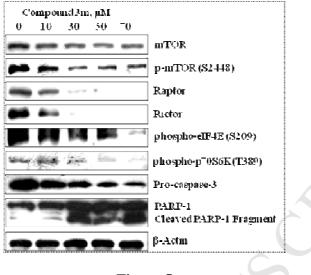
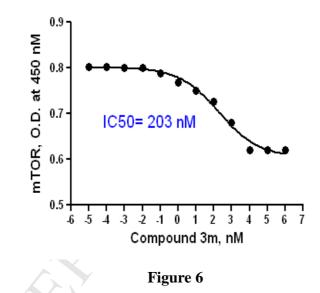
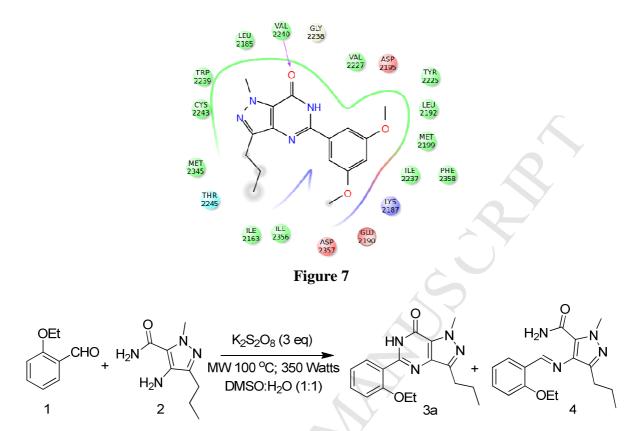


Figure 5





Scheme 1: Microwave assisted synthesis of 5-(2-ethoxyphenyl)-1-methyl-3-propyl-1Hpyrazolo[4,3-d]pyrimidin-7(6H)-one

Highlights

Synthesis of 5-substituted-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one analogs and their biological evaluation as anticancer agents: mTOR inhibitors

G. Lakshma Reddy, Santosh Kumar Guru, M. Srinivas, Anup Singh Pathania, Priya Mahajan, Amit Nargotra, Shashi Bhushan,* Ram A. Vishwakarma,* Sanghapal D. Sawant*

<u>Highlights</u>

- Microwave assisted synthesis of 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one scaffold
- In vitro anticancer activity: HeLa, CAKI-I, PC-3, MiaPaca-2, A549 cancer cell lines
- Compound **3m:** cell cycle analysis, western blotting, nuclear cell morphology
- mTOR inhibitory potential of compound **3m** with nonomolar potency: $IC_{50}= 203 \text{ nM}$

Appendix

Supplementary data

Synthesis of 5-substituted-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one analogs and their biological evaluation as anticancer agents: mTOR inhibitors

G. Lakshma Reddy,^{a,d} Santosh Kumar Guru,^b M. Srinivas,^{a,d} Anup Singh Pathania,^{b,d} Priya Mahajan,^{c,d} Amit Nargotra,^{c,d} Shashi Bhushan,^{b,d}* Ram A. Vishwakarma,^{a,d,*} Sanghapal D. Sawant,^{a,d,*}

^aMedicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Canal Road-Jammu-180 001, India ^bCancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road-Jammu-180 001, India ^cDiscovery Informatics, CSIR-Indian Institute of Integrative Medicine, Canal Road-Jammu-180 001, India ^dAcademy of Scientific and Innovative Research, India Tel.: +91-191-2569111, Fax: +91-191-2569333; IIIM Communication No. IIIM/1610/2013 E-mail: sdsawant@iiim.ac.in , sawant.rrl@gmail.com

Table of Contents

S.No	Content	Page Nos
1.1.	Table 1: Screening of catalyst for oxidative	S 3
	cyclization and formation of 3a	
1.2.	Table 2: Solvent screening for the formation of 3a	S3
1.3.	Table 3: Optimization of microwave protocols for	S4
	the synthesis of 3a	
1.4.	Analytical data	S4-S7
1.5.	Scanned spectral data of compounds	S8-S61
	References	S62

S.No	Solvent	Oxidizing	Yield	(%)
		agent	3 a	4
1	DMSO/H ₂ O(1:1)	H_2O_2	0	75
2	DMSO/H ₂ O(1:1)	TBHP	0	70
3	DMSO/H ₂ O(1:1)	<i>m</i> -CPBA	0	75
4	DMSO/H ₂ O(1:1)	Oxone	15	15
5	DMSO/H ₂ O(1:1)	K ₂ S ₂ O ₈	98	0
5	DMSO/H ₂ O(1:1)	K ₂ S ₂ O ₈	98	0

1.1. Table 1: Screening of catalyst for oxidative cyclization and formation of 3a

1.2. Table 2: Solvent screening for the formation of 3a

Sr. No	Solvent	Oxidant	Yiel	d(%) ^a
			<u>3a</u>	4
1	EtOH		0	95
2	ACN		0	90
3	THF		0	85
4	PhCl		0	50
5	H ₂ O		20	45
6	EtOH/H ₂ O(1:1)	$K_2S_2O_8$	20	75
7	ACN/H ₂ O(1:1)		85	10
8	THF/H ₂ O(1:1)		65	30
9	DMF		85	10
10	DMSO		90	5
11	DMF/H ₂ O(1:1)		90	5
12	DMSO/H ₂ O(1:1)		98	0

^aAll yields are isolated yields after column purification

Easter :	Microwave	Temp	Time	Yield	l(%) ^a
Entry	Power (Watts)	(°C)	(Min)	3a	4
		(a) 80 -	3	20	75
1	100	(u) 00	5	25 70 35 60	70
1	100	(b) 100	3		60
		(0) 100	5	40	55
			3	40	50
2	250 -	(a) 80	5	45	50
2	230 -	(b) 100 .	3	35 40 40 45 55	40
		(0) 100	5	65	30
		(a) 80	3	80	15
3	350	(a) 80	5	85	10
		(b) 100	3	98	0

1.3. Table 3: Optimization of microwave protocols for the synthesis of 3a

^aAll yields are isolated yields after column purification

1.4. Analytical data

3a) 5-(2-ethoxyphenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one**¹**:** ¹H NMR (400 MHz, CDCl₃) δ 11.14 (s, 1H), 8.48 (d, *J* = 8.0 Hz, 1H), 7.46 (m 1H), 7.15 (m 1H), 7.04 (d, *J* = 8.4Hz, 1H), 4.35 – 4.23 (m, 5H), 2.95 (t, *J* = 7.6 Hz, 2H), 1.95 – 1.83 (m, 2H), 1.61 (t, *J* = 7.2 Hz, 3H), 1.05 (t, *J* = 7.2Hz, 3H). HRMS (ESI) calcd for C₁₇H₂₁N₄O₂ ([M-H⁺] 313.16645, found 313.16580.

3b) 5-(4-methoxyphenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one^{1,3}: ¹H NMR (400 MHz, CDCl₃) \delta 10.71 (s, 1H), 8.06 (d,** *J* **= 8.8Hz, 2H), 7.02 (d,** *J* **= 8.8 Hz, 2H), 4.28 (s, 3H), 3.89 (s, 3H), 2.93 (t,** *J* **= 7.6 Hz, 2H), 1.93 – 1.82 (m, 2H), 1.03 (t,** *J* **= 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₆H₁₉N₄O₂ ([M-H⁺] 299.15080, found 299.15035**

3c) (E)-1-methyl-3-propyl-5-styryl-1*H*-pyrazolo [4, 3-d] pyrimidin-7(6*H*)-one: ¹H NMR (400 MHz, CDCl₃) δ 11.50 (s, 1H), 7.75 (d, *J* = 16.4Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.46 – 7.34 (m, 3H), 6.97 (d, *J* = 16.4 Hz, 1H), 4.29 (s, 3H), 2.92 (t, *J* = 7.6 Hz, 2H), 1.92 – 1.80 (m, 2H), 1.04 (t, *J* = 7.2 Hz, 3H).¹³C NMR (126 MHz, DMSO) δ 154.15, 149.09, 144.71, 137.97, 136.22,

135.16, 129.31, 128.95, 127.31, 124.28, 120.99, 37.77, 27.09, 21.65, 13.85. HRMS (ESI) calcd for $C_{17}H_{19}N_4O$ [M-H⁺] 295.15589, found 295.15622.

3d) 5-(3-bromo-4-methoxyphenyl)-1-methyl-3-propyl-1*H*-pyrazolo [4, 3-d] pyrimidin-7(6*H*)-one: ¹H NMR (400 MHz, DMSO) δ 11.80 (s, 1H), 8.26 (d, *J* = 1.8 Hz, 1H), 8.05 (dd, *J* = 8.8, 1.7 Hz, 1H), 7.18 (d, *J* = 8.8 Hz, 1H), 4.08 (s, 3H), 3.86 (s, 3H), 2.73 (t, *J* = 7.6 Hz, 2H), 1.77 - 1.62 (m, 2H), 0.89 (t, *J* = 7.2 Hz, 3H). HRMS (ESI) calcd for C₁₆H₁₈BrN₄O₂ [M-H⁺] 377.06131, found 377.05963.

3e) 1-methyl-5-(3-nitrophenyl)-3-propyl-1*H*-pyrazolo [4, 3-d] pyrimidin-7(6*H*)-one¹: ¹H NMR (400 MHz, DMSO) δ 8.90 (s, 1H), 8.50 (d, *J* = 8.0 Hz, 1H), 8.38 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 4.17 (s, 3H), 2.82 (t, *J* = 7.4 Hz, 2H), 1.85 – 1.71 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₅H₁₆N₅O₃ [M-H⁺] 314.12531, found 314.12430.

3f) 5-(3-bromo-4-fluorophenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)one: ¹H NMR (400 MHz, DMSO) \delta 12.51 (s, 1H), 8.41 (d,** *J* **= 5.2 Hz, 1H), 8.13 (s, 1H), 7.54 (m 1H), 4.16 (s, 3H), 2.81 (t,** *J* **= 7.5 Hz, 2H), 1.84 – 1.69 (m, 2H), 0.96 (t,** *J* **= 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₅H₁₅BrFN₄O [M-H⁺] 365.04133, found 365.04088.**

3g) 1-methyl-5-(5-nitrofuran-2-yl)-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one: ¹H NMR (400 MHz, DMSO) \delta 8.32 (s, 1H), 7.88 (s, 1H), 4.16 (s, 3H), 2.82 (t,** *J* **= 7.4 Hz, 2H), 1.84 – 1.68 (m, 2H), 0.96 (t,** *J* **= 7.3 Hz, 3H). ¹³C NMR (101 MHz, DMSO) \delta 153.75, 152.07, 147.43, 145.67, 139.96, 136.88, 124.72, 115.02, 114.06, 37.92, 27.02, 21.52, 13.79. HRMS (ESI) calcd for C₁₃H₁₄N₅O₄ [M-H⁺] 304.10458, found 304.10409.**

3h) 5-(4-hydroxyphenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one**¹**:** ¹H NMR (400 MHz, DMSO) δ 12.16 (s, 1H), 10.04 (s, 1H), 7.95 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 4.14 (s, 3H), 2.79 (t, J = 7.5 Hz, 2H), 1.88 – 1.62 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₅H₁₇N₄O₂ [M-H⁺] 285.13515, found 285.13382.

3i) 5-(4-chlorophenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one**^{2, 3}**:** ¹H NMR (400 MHz, CDCl₃) δ 10.94 (s, 1H), 8.08 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 4.30 (s, 3H), 2.93 ((t, *J* = 7.6 Hz, 3H), 1.92 – 1.81 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₅H₁₆ClN₄O [M-H⁺] 303.10126, found 303.0991.

3j) 1-methyl-3-propyl-5-(2,4,5-trimethoxyphenyl)-1*H*-pyrazolo[4,3-d]pyrimidin-7(6*H*)-one: ¹H NMR (400 MHz, CDCl₃) δ 10.99 (s, 1H), 8.03 (s, 1H), 6.59 (s, 1H), 4.27 (s, 3H), 4.05 (s, 3H), 3.97 (s, 6H), 2.93 (t, J = 7.5 Hz, 2H), 1.93 – 1.83 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 153.61, 152.09, 151.83, 149.06, 144.66, 142.60, 137.96, 123.88, 113.50, 112.81, 97.89, 56.57, 56.15, 55.89, 37.77, 27.07, 21.59, 13.81. HRMS (ESI) calcd for C₁₈H₂₃N₄O₄ [M-H⁺] 359.17193, found 359.17139.

3k) 5-(4-methoxy-3-nitrophenyl)-1-methyl-3-propyl-1*H*-pyrazolo [4, 3-d] pyrimidin-7(6*H*)one: ¹H NMR (400 MHz, DMSO) δ 12.54 (s, 1H), 8.62 (d, *J* = 2.3 Hz, 1H), 8.38 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.52 (d, *J* = 9.0 Hz, 1H), 4.16 (s, 3H), 4.01 (s, 3H), 2.81 (t, *J* = 7.5 Hz, 2H), 1.83 – 1.71 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₆H₁₈N₅O₄ [M-H⁺] 344.13588, found 344.13441.

3l) 5-(3-fluoro-4-methoxyphenyl)-1-methyl-3-propyl-1*H*-pyrazolo [4, 3-d] pyrimidin-7(6*H*)one: ¹H NMR (400 MHz, CDCl₃) δ 11.36 (s, 1H), 8.07 (dd, *J* = 10.8 ,1.8Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.07 (t, *J* = 8.4 Hz, 1H), 4.31 (s, 3H), 3.98 (s, 3H), 2.93 (t, *J* = 7.6 Hz, 2H), 1.96 – 1.76 (m, 2H), 1.04 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (101 MHz, DMSO) δ 154.55, 152.26, 149.25, 148.61, 144.85, 137.76, 125.44, 124.26, 114.90, 114.70, 113.57, 56.20, 37.80, 27.09, 21.61, 13.83; HRMS (ESI) calcd for C₁₆H₁₈FN₄O₂ [M-H⁺] 317.14138, found 317.14025.

3m) 5-(3,5-dimethoxyphenyl)-1-methyl-3-propyl-1*H***-pyrazolo[4,3-d]pyrimidin-7(6***H***)-one: ¹H NMR (400 MHz, CDCl₃) \delta 10.92 (s, 1H), 7.25 (d,** *J* **= 2.0 Hz, 2H), 6.61 (t,** *J* **= 2.0 Hz, 1H), 4.30 (s, 3H), 3.89 (s, 6H), 2.98 – 2.89 (m, 2H), 1.93 – 1.80 (m, 2H), 1.03 (t,** *J* **= 7.4 Hz, 3H). ¹³C (126 MHz, CDCl₃) \delta 161.27, 155.58, 149.29, 146.87, 139.14, 134.88, 124.55, 105.53, 102.65, 55.60, 38.18, 27.73, 22.35, 14.05 HRMS (ESI) calcd for C₁₇H₂₁N₄O₃ [M-H⁺] 329.16137, found 329.16031.**

3n) 5-(3-chlorophenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one³:** ¹H NMR (400 MHz, CDCl₃) δ 11.76 (s, 1H), 8.23 (s, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.54 – 7.43 (m, 2H), 4.33 (s, 3H), 2.94 (t, *J* = 7.6 Hz, 2H), 1.93 – 1.82 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₅H₁₆ClN₄O [M-H⁺] 303.10126, found 303.09995.

3o) 5-(2-fluorophenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one:** ¹H NMR (400 MHz, CDCl₃) δ 9.73 (d, *J* = 10.4 Hz, 1H), 8.34-8.28 (m, 1H), 7.51 (dd, *J* = 13.7, 7.1 Hz, 1H), 7.37-7.31 (m,1H), 7.25-7.18 (m, 1H), 4.27 (s, 3H), 2.92 (d, *J* = 7.6 Hz, 2H), 1.92 – 1.81 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO) δ 160.70, 158.22, 154.01,

147.04, 144.99, 137.70, 132.39, 131.01, 124.52, 122.27, 116.13, 37.83, 27.09, 21.66, 13.76. HRMS (ESI) calcd for $C_{15}H_{16}FN_4O$ [M-H⁺] 287.13081, found 287.12944.

3p) 5-(3-fluorophenyl)-1-methyl-3-propyl-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one⁴:** ¹H NMR (400 MHz, CDCl₃) δ 11.71 (s, 1H), 8.05 -7.87(m, 2H), 7.54-7.46 (m, 1H), 7.26-7.20 (m, 1H), 4.31 (s, 3H), 2.94 (t, *J* = 7.6 Hz, 2H), 1.93 – 1.82 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₅H₁₆FN₄O [M-H⁺] 287.13081, found 287.1304.

3q) 5-(4-hydroxy-3-nitrophenyl)-1-methyl-3-propyl-1*H***-pyrazolo[4,3-d]pyrimidin-7(6***H***)one: ¹H NMR (400 MHz, DMSO) \delta 12.49 (s, 1H), 8.64 (s, 1H), 8.25 (d,** *J* **= 8.0 Hz, 1H), 7.24 (d,** *J* **= 8.0 Hz, 1H), 4.14 (s, 3H), 2.79 (t,** *J* **= 7.5 Hz, 2H), 1.86 – 1.65 (m, 2H), 0.95 (t,** *J* **= 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO) \delta 154.47, 153.82, 148.10, 144.86, 137.62, 136.67, 133.76, 124.47, 123.80, 119.21, 79.11, 37.78, 27.08, 21.57, 13.82. HRMS (ESI) calcd for C₁₅H₁₆N₅O₄ [M-H⁺] 330.12023, found 330.11878.**

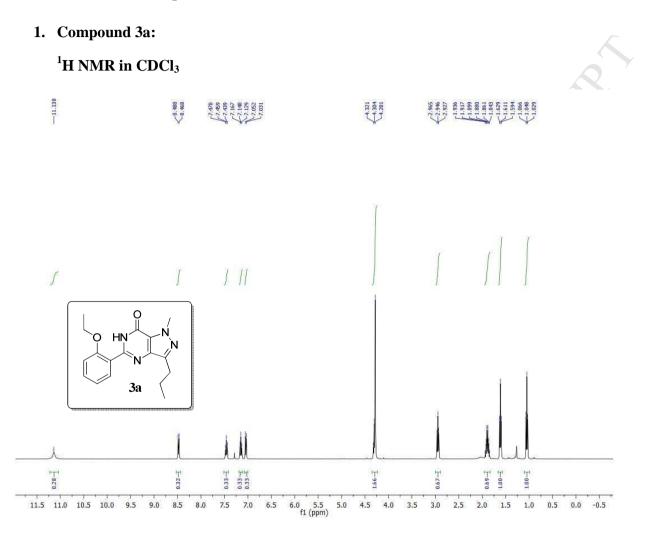
3r) 5-(3, 4-dimethoxyphenyl)-1-methyl-3-propyl-1*H***-pyrazolo[4,3-d]pyrimidin-7(6***H***)-one²: 1H NMR (400 MHz, CDCl₃) \delta 11.03 (s, 1H), 7.69 – 7.62 (m, 2H), 6.95 (d,** *J* **= 8.4 Hz, 1H), 4.26 (s, 3H), 4.01 (s, 3H), 3.95 (s, 3H), 2.93 (t,** *J* **= 7.6 Hz, 2H), 1.93 – 1.80 (m, 2H), 1.03 (t,** *J* **= 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₇H₂₁N₄O₃ [M-H⁺] 329.16137, found 329.16032.**

3s) 1-methyl-3-propyl-5-(pyridin-3-yl)-1*H***-pyrazolo [4, 3-d] pyrimidin-7(6***H***)-one**¹**:** ¹H NMR (400 MHz, CDCl₃) δ 12.13 (s, 1H), 9.43 (d, *J* = 1.8 Hz, 1H), 8.78 (dd, *J* = 4.7, 1.1 Hz, 1H), 8.58–8.43 (m, 1H), 7.47 (dd, *J* = 7.9, 4.8 Hz, 1H), 4.32 (s, 3H), 2.95 (t, *J* = 7.6 Hz, 2H), 1.95 – 1.82 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₄H₁₆N₅O [M-H⁺] 270.13549, found 270.13503.

3t) **5-(benzo[d][1,3]dioxol-5-yl)-1-methyl-3-propyl-1***H***-pyrazolo[4,3-d]pyrimidin-7(6***H***)-one:** ¹H NMR (400 MHz, DMSO) δ 12.25 (s, 1H), 7.67 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.62 (d, *J* = 1.7 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.12 (s, 2H), 4.14 (s, 3H), 2.79 (t, *J* = 7.5 Hz, 2H), 1.83 – 1.70 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) calcd for C₁₆H₁₇N₄O₃ [M-H⁺] 313.13007, found 313.12878.

ACCEPTED MANUSCRIPT

1.2. Scanned Spectral Data:



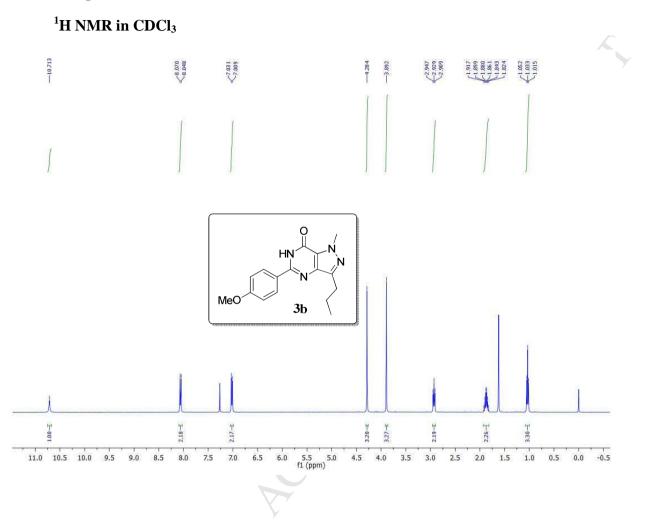
HRMS

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	_			Qua	alitat	tive Con	ipound	Report			-
Data File Sample Type Instrument N Acq Method RM Calibrati Comment Sample Group	on SI	atus Info	Succes	e nent 1 MS_2507201	2.m	Sample Name Position User Name Acquired Time DA Method	GLR-OEt Vial 22 11/19/201 as.m	2 2:01:46 PM			o N 3a
Compound ⁻	Table	8	_						MEGD		
Compour	dia	hel	RT	Mass		Formula	ME	G Formula	(ppm		B Formula
Cpd 19: 0			0.171	312.15852	C	17 H20 N4 O2		7 H20 N4 O2			7 H20 N4 C
Compound Cpd 19: C17			<i>m/z</i> 313.1658	RT 0.171	Algorit Find by	hm Molecular Featu	Mass re 312.1585	2			
5 - 4 - 3 -			*313.16580 C17 H91 M4 00				647.30494 H40 N8 Na O4				
2 1 0	150	200 2	50 300	350 400	450 50		C34	750 800 850	900 95	0	
1-	150 m P		50 300	350 400 Col	450 50 unts vs.	00 550 600 Mass-to-Charg	650 700	750 800 850	900 95	50	
MS Spectru	m Po	ak List Abund	Form	Co	450 50 unts vs.	00 550 600 Mass-to-Charg	650 700	750 800 850	900 95	50	
1 0 MS Spectru m/z 313.1658	m Po	ak List Abund 6190	Form 0320 C17 H	Col ula 121 N4 O2	450 50 unts vs.	00 550 600 Mass-to-Charg Ion (M+H)+	650 700	750 800 850	900 95	.0	
1 0 MS Spectru m/z 313.1658 314.16901	m P z 1	Abund 6190 11787	Form 0320 C17 H 16.9 C17 H	Col ula 121 N4 O2 121 N4 O2	450 50 unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+	650 700	750 800 850	900 95	0	
1 0 MS Spectru m/z 313.1658 314.16901 315.17101	m Po	eak List Abund 6190 11787 1326	Form 0320 C17 H 16.9 C17 H 20.6 C17 H	Col ula 121 N4 O2 121 N4 O2 121 N4 O2	450 50 unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+ (M+H)+	650 700	750 800 850	900 95	0	
1- 0 MS Spectru <i>m/z</i> 313.1658 314.16901 315.17101 316.17283	z 1 1 1 1 1	eak List Abund 6190 11787 1326	Form 0320 C17 H 216.9 C17 H 20.6 C17 H 9972 C17 H	Col 21 N4 O2 21 N4 O2 21 N4 O2 21 N4 O2 21 N4 O2	unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+	650 700	750 800 850	900 95	0	
1- 0- MS Spectru <i>m/z</i> 313.1658 314.16901 315.17101 316.17283 335.14715	m Po z 1 1 1 1 1	Abund 6190 11787 1326 9 1196	Form 0320 C17 H 16.9 C17 H 20.6 C17 H 9972 C17 H 519.7 C17 H	Col ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2	unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+	650 700	750 800 850	900 95	0	
MS Spectru m/z 313.1658 314.16901 315.17101 316.17283 335.14715 336.15019	m Po z 1 1 1 1 1 1 1	eak List Abund 6190 11787 1326 9 1196 222	Form 0320 C17 H 16.9 C17 H 20.6 C17 H 9972 C17 H 19.7 C17 H 15.4 C17 H	Con ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 N4 Na O2	unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+	650 700	750 800 850	900 95	50	
MS Spectru m/z 313.1658 314.16901 315.17101 316.17283 335.14715 336.15519 351.12206	m Po z 1 1 1 1 1 1 1 1	eak List Abund 6190 11787 1326 1196 222 30	Form 0320 C17 H '16.9 C17 H 20.6 C17 H 9972 C17 H 19.7 C17 H 21.5.4 C17 H 19.9.2 C17 H	Con ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 K N4 O2	unts vs.	Ion [Mass-to-Charg [M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+ (M+K)+	650 700	750 800 850	900 95	00	
MS Spectru m/z 313.1658 314.16901 315.17101 316.17283 335.14715 336.15019 351.12206 647.30494	m Po z 1 1 1 1 1 1 1 1 1 1	eak List Abund 6190 11787 1326 1196 222 30 293	Form 0320 C17 + '16.9 C17 + '20.6 C17 + '9972 C17 + '115.4 C17 + '125.4 C17 + '13.8 C34 +	Col ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 K N4 O2 140 N8 Na O4	unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+K)+ (M+K)+ (2M+Na)+ (M+K)+ (M+K)+	650 700	750 800 850	900 95	0	
MS Spectru m/z 313.1658 314.16901 315.17101 316.17283 335.14715 336.15019 351.12206 648.30728	m Po z 1 1 1 1 1 1 1 1 1 1 1 1 1	eak List Abund 6190 11787 1326 1196 222 300 293 1300	Form 0320 C17 H '16.9 C17 H '20.6 C17 H '19.7 C17 H '15.4 C17 H '15.4 C17 H '15.4 C17 H '13.8 C34 H '061.5 C34 H	Col ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 K N4 O2 1	unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (2M+Na)+ (2M+Na)+	650 700	750 800 850	900 95	0	
MS Spectru m/z 313.1658 314.16901 315.17101 316.17283 335.14715 336.15019 351.12206 647.30494 648.30728 649.31084	m Po z 1 1 1 1 1 1 1 1 1 1 1 1 1	eak List Abund 6190 11787 1326 1196 222 300 293 1300	Form 0320 C17 H 116.9 C17 H 120.6 C17 H 1972 C17 H 115.4 C17 H 199.7 C17 H 115.4 C17 H 115.4 C17 H 113.8 C34 H 161.5 C34 H 3209 C34 H	Col ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 K N4 O2 140 N8 Na O4	unts vs.	00 550 600 Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+K)+ (M+K)+ (2M+Na)+ (M+K)+ (M+K)+	650 700	750 800 850	900 95	0	
MS Spectru m/z 313.1658 314.1690 315.17101 315.17101 335.14715 336.15019 335.14715 336.15019 351.12206 647.30494 648.30728 649.31084 Predicted I	m P 2 1 1 1 1 1 1 1 1 1 1 1 1 1	eak List Abund 6190 11787 1326 222 300 293 130 pe Mate	Form 0320 C17 H '16.9 C17 H '20.6 C17 H '9972 C17 H '15.4 C17 H '15.4 C17 H '94.9 C14 H '94.9 C34 H '94.9 C34 H	Col ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 N N4 O2 120 N N4 O2 140 N8 Na O4 140 N8 Na O4 140 N8 Na O4		00 550 600 Mass-to-Charg Image: Constraint of the second sec	650 700	750 800 850		ic Abund Sum	%
MS Spectru m/z 313.1658 314.1690 315.17101 316.17283 335.14715 336.15019 351.12206 647.30494 648.30728 649.31084 Predicted I Isotope	m Po z 1 1 1 1 1 1 1 1 1 1 1 1 1	eak List Abund 619(11787 1326 2222 30 293 130 pe Mate	Form 0320 C17 + '16.9 C17 + '20.6 C17 + '972 C17 + '115.4 C17 + '115.4 C17 + '13.8 C34 + '61.5 C34 +	Col ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 K N4 O2 1		00 550 600 Mass-to-Charg Image: Constraint of the second sec	850 700 3 ∍ (m/z)	Abund Sum %			% 81.49
MS Spectru m/z 313.1658 314.16901 315.17101 316.17283 335.14715 336.15019 351.12206 647.30494 648.30728 649.31084 Predicted I Isotope	m Po z 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	eak List Abund 619(11787 1326 9 1196 222 300 293 130 293 130 293 130 293 130 293 130 293 130 293 130 293	Form 0320 C17 + 16.9 C17 + 20.6 C17 + 1972 C17 + 1973 C17 + 1974 C17 + 1975 C17 + 115.4 C17 + 194.9 C17 + 13.8 C34 + 143.8 C34 + 13.8 C34 + 13.3 C34 + 14 Table Calc m/z 313.1	Con ula 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 121 N4 O2 120 N4 Na O2 120 N4 Na O2 120 K N4 O2 140 N8 Na O4 140 N8 N	m) Ab	Ion Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+ (M+Na)+ (M+Ka)+ (M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+	650 700 ° e (m/z) Abund %	Abund Sum %	Cal		81.49 16.43
MS Spectru m/z 313.1658 314.1690 315.17101 316.17283 335.14715 336.15019 351.12206 647.30494 648.30728 649.31084 Predicted I Isotope 1 2	m Po z 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	eak List Abund 619(11787 1326 222 30(293) 1306 293 133(5) 5) 5) 6) 6) 7) 7) 7) 7) 7) 7) 7) 7) 7) 7) 7) 7) 7)	Form 0320 C17 + '16.9 C17 + '20.6 C17 + '972 C17 + '115.4 C17 + '115.4 C17 + '13.8 C34 + '61.5 C34 +	ula 121 N4 02 120 N4 Na 02 120 N4 Na 02 120 N4 Na 04 140 N8 Na 04 Diff (pp 659 884	m) Ab	Ion Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ 100	850 700 ° ₂ (m/z)	Abund Sum %	Ca 82.41		81.49
MS Spectru m/z 313.1658 314.16901 315.17101 316.17283 335.14715 336.15019 351.12206 647.30494 648.30728 649.31084 Predicted I Isotope	m P(z 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 5 5 5 5	eak List Abund 619(11787 1326 9 1196 222 300 293 130 293 130 293 130 293 130 293 130 293 130 293 130 293	Form 0320 C17 F 16.9 C17 F 20.6 C17 F 9972 C17 F 115.4 C17 F 115	Ula 121 N4 02 120 N4 Na 02 120 N4 Na 02 120 N4 Na 02 120 N4 Na 02 140 N8 Na 04 140 N8 Na 04 Diff (pp 659 884 147	m) Ab 0.34 -0.55	00 550 600 Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ (2M+Na)+ und % Calc 100 19.04 19.04 Calc	650 700 (m/z) Abund % 100 20.17	Abund Sum %	Cal 82.41 15.69		81.49 16.43

--- End Of Report ---

2. Compound 3b

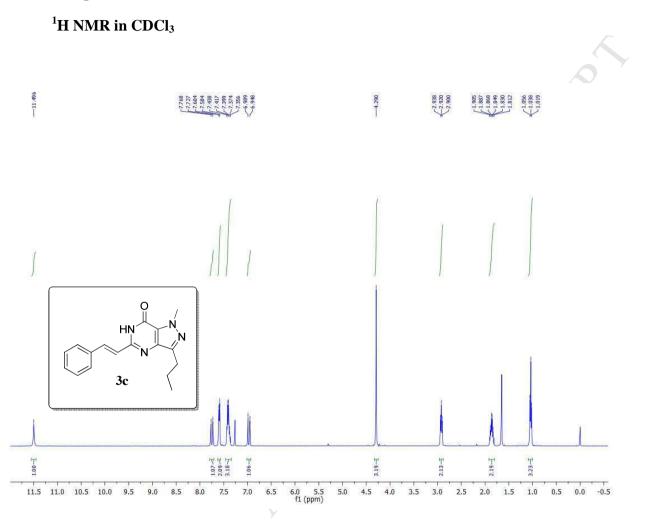


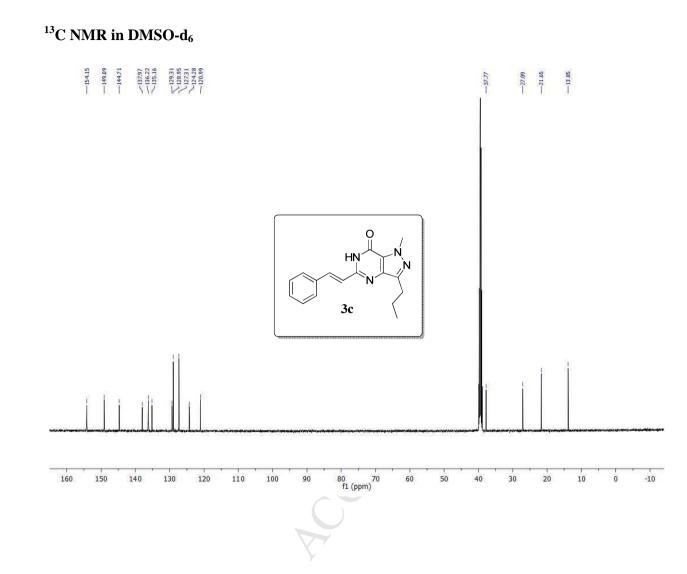
HRMS

Qualitative Compound Report O Data File GLR-08.d Sample Name Unavailable Unavailable Position Unavailable HN Sample Type Unavailable User Name Unavailable Instrument Name Acquired Time Unavailable Acq Method N DA Method **IRM Calibration Status** as.m Sample information is unavailable Comment MeO 3b Compound Table MEG Diff Compound Label Cpd 7: C16 H18 N4 O2 RT MFG Formula Mass Formula **DB** Formula (ppm) 0.171 298.14307 C16 H18 N4 O2 C16 H18 N4 O2 -0.33 C16 H18 N4 O2 **Compound Label** RT Algorithm Mass m/z Cpd 7: C16 H18 N4 O2 299.15035 0.171 Find by Molecular Feature 298.14307 MFE MS Spectrum x10 6 Cpd 7: C16 H18 N4 O2: + MFE Spectrum (0.114-0.507 min) GLR-08.d 299.15035 C16 H19 N4 O2 1.2 1 0.8 0.6 0.4 0.2 0 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 Counts vs. Mass-to-Charge (m/z) **MS Spectrum Peak List** z Abund Formula Ion m/z 299.15035 1 1390353.4 C16 H19 N4 O2 (M+H)+ 300.15282 1 224317 C16 H19 N4 O2 (M+H)+ 301.15497 1 26806.6 C16 H19 N4 O2 (M+H)+ 302.1558 1 3087.3 C16 H19 N4 O2 (M+H)+ 321.13218 1 6009.2 C16 H18 N4 Na O2 (M+Na)+ 322.13399 1 1351.4 C16 H18 N4 Na O2 (M+Na)+ Predicted Isotope Match Table Calc Abund % Abund Sum % Calc Abund Sum % Isotope m/z Calc m/z Diff (ppm) Abund % 299.15035 84.54 82.39 299.15025 -0.34 100 100 300.15282 300.15316 16.13 19.06 13.64 15.71 1.12 1.76 1.63 301.15497 2.56 1.93 2.13 3 301.15574 0.19 0.14 302.1558 8.02 0.22 0.18 4 302.15822

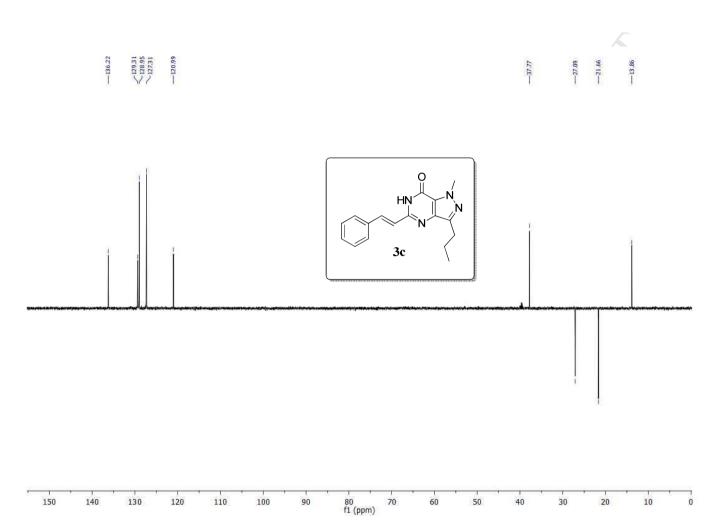
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3. Compound 3c







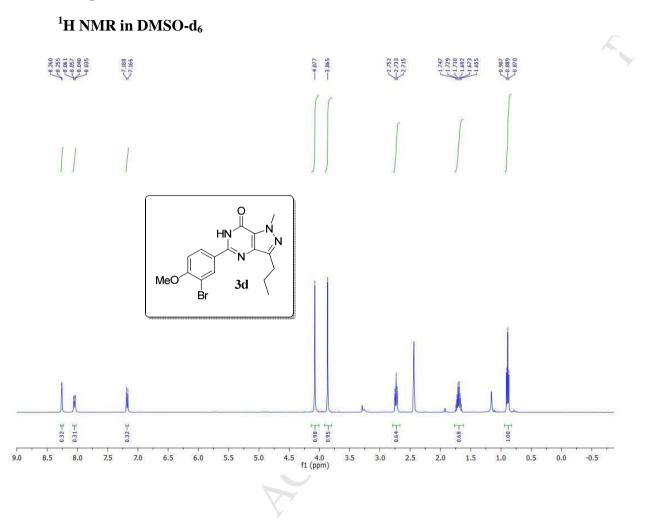


Qualitative Compound Report

Sample Type Instrument N Acq Method IRM Calibrat Comment Sample Grou Compound	Name ion Status P	s Info.	Sample Instrume	IS DESI.m	Sample Nam Position User Name Acquired Tin DA Method	57	12 5:09:29 PM			
			-						FG Diff	
Compou	C17 H18 N		RT 0.338	Mass 294.14893	Formula C17 H18 N4 O		MFG Formula	- (ppm)	DB Formula
сра 75.	G17 110 N		0.000	254.14055	C17 110 14 U		L17 118 N4 U	_	-2.96	C17 H18 N4 O
								-	1	
Compound		m/.		RT	Algorithm		ISS			
Cpd 73: C17	H18 N4 O	295	.15622	0.338	Find by Molecular	Feature 294	4.14893			
3.5 - 3 - 2.5 - 2 -			295.15622 C17 H19 N4 O							
3 2.5 2 1.5 1 0.5 0 15		250	295.15622 00 C17 H19 N4 (350 400 Counts	450 500 55 vs. Mass-to-Charg	50 600 (je (m/z)	650 700 75	50 80	10	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrur	n Peak L	ist	300	Counts	vs. Mass-to-Charg	50 600 (je (m/z)	650 700 75	50 8C	00	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrur	n Peak L z Abun	ist d		Counts	vs. Mass-to-Charg	50 600 (je (m/z)	650 700 75	50 80	00	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum m/z	n Peak L z Abun 1 4	ist d 28039.7	300 Formula	Counts N4 O	vs. Mass-to-Charg	50 600 (ge (m/z)	650 7 <u>00</u> 75	50 80	00	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum m/z 295.15622	n Peak L z Abun 1 4	ist d 28039.7 03269.4	300 Formula C17 H19	Counts N4 O N4 O	vs. Mass-to-Charg	50 600 (je (m/z)	650 700 75	50 80	00	
3 2.5 2 1.5 1 0.5 0 15 0 15 0 15 0 15 0 15 0 15	n Peak L z Abun 1 4 1 1	ist d 28039.7 03269.4 9196.2	300 Formula C17 H19 C17 H19	Counts N4 0 N4 0 N4 0 N4 0	vs. Mass-to-Charg Ion (M+H)+ (M+H)+	50 600 (je (m/z)	650 700 75	50 80	00	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum <i>m/z</i> 295.15622 296.15817 297.16111	m Peak L z Abun 1 4 1 1 1	ist d 28039.7 03269.4 9196.2 858.2	300 Formula C17 H19 C17 H19 C17 H19	Counts N4 0 N4 0 N4 0 N4 0 N4 0	Vs. Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+	50 600 (e (m/z)	550 700 75	60 8C	00	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum <i>m/z</i> 295.15622 296.15817 297.16111 298.16345	z Abun 1 4 1 1 1 1 1 1	ist 28039.7 03269.4 9196.2 858.2 3055.8	300 Formula C17 H19 C17 H19 C17 H19 C17 H19 C17 H19	Counts N4 0 N4 0 N4 0 N4 0 K N4 0	Vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+	50 600 (je (m/z)	550 700 75	60 8C	0	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum m/z 295.15622 296.15817 297.16111 298.16345 333.11103 334.11312 335.1149	z Abun 1 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	ist d 28039.7 03269.4 9196.2 858.2 3055.8 521.5 341.6	300 Formula C17 H19 C17 H19 C17 H19 C17 H19 C17 H18 C17 H18 C17 H18	Counts N4 0 N4 0 N4 0 N4 0 K 0 K N4 0 K N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+	50 600 (je (m/z)	550 700 75	60 80	0	
3 2.5 2 1.5 1 0.5 0 MS Spectrum <i>m/z</i> 295.15622 296.15817 297.16111 298.16345 333.11103 334.11312 335.1149 Predicted Is	n Peak L z Abun 1 4 1 1 1 1 1 1 1 1 1 1 1 1 1 0	ist d 28039.7 03269.4 9196.2 858.2 3055.8 521.5 341.6 atch Ta	300 Formula C17 H19 C17 H19 C17 H19 C17 H19 C17 H18 C17 H18 C17 H18 C17 H18 ble	Counts N4 0 N4 0 N4 0 N4 0 K N4 0 K N4 0 K N4 0	vs. Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+ (M+K)+ (M+K)+	je (m/z)	650 700 75	50 80	00	
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum m/z 295.15622 296.15817 297.16111 298.16345 333.11103 334.11312 335.1149 Predicted Is Isotope	m Peak L z Abun 1 4 1 1 1 1 1 1 1 1 0tope Ma m/z	ist d 28039.7 03269.4 9196.2 858.2 3055.8 521.5 341.6 atch Ta Calc	300 Formula C17 H19 C17 H19 C17 H19 C17 H19 C17 H18 C17 H18 C17 H18 C17 H18 ble m/z	Counts N4 0 N4 0 N4 0 N4 0 K N4 0 K N4 0 K N4 0 K N4 0 Diff (ppm)	vs. Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+ (M+K)+ (M+K)+ Abund % Calc	0 600 ge (m/z)	650 700 75	50 80	00 Calc Abund	Sum %
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum m/z 295.15622 295.15622 295.15622 295.15622 295.15622 333.11103 333.11103 333.11103 333.11102 Biotope 1	n Peak L z Abun 1 4 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ist d 28039.7 03269.4 9196.2 858.2 3055.8 521.5 341.6 atch Ta Calc 522 2	300 Formula C17 H19 C17 H19 C17 H19 C17 H19 C17 H18 C17 H18 C17 H18 ble sm/z 95.15534	Counts N4 0 N4 0 N4 0 N4 0 K N4 0 K N4 0 K N4 0 K N4 0 Diff (ppm)	vs. Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+ (M+K)+ (M+K)+ Abund % Calc	je (m/z)	Abund Sum %	50 80		Sum % 81.71
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum m/z 295.15622 296.15817 297.16111 298.16345 333.11103 334.11312 335.1149 Predicted IS Isotope 1 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2	m Peak L z Abun 1 4 1 1 1 1 1 1 1 1 0tope Ma m/z	ist d 28039.7 03269.4 9196.2 858.2 3055.8 521.5 341.6 atch Ta Calc 522 2	300 Formula C17 H19 C17 H19 C17 H19 C17 H19 C17 H18 C17 H18 C17 H18 C17 H18 c17 H18 ble m/z	Counts N4 0 N4 0 N4 0 N4 0 K N4 0 K N4 0 K N4 0 K N4 0 Diff (ppm)	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+H)+ (M+	ge (m/z)	Abund Sum %			
3 2.5 2 1.5 1 0.5 0 15 MS Spectrum m/z 295.15622 295.15622 295.15622 295.15622 295.15622 333.11103 333.11103 333.11103 333.11102 Biotope 1	n Peak L z Abun 1 4 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ist d 28039.7 03269.4 9196.2 858.2 3055.8 521.5 341.6 atch Ta Calc 522 2 317 2	300 Formula C17 H19 C17 H19 C17 H19 C17 H19 C17 H18 C17 H18 C17 H18 ble sm/z 95.15534	Counts N4 0 N4 0 N4 0 N4 0 N4 0 K N4 0 K N4 0 K N4 0 K N4 0 Diff (ppm) -2.99	Vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+H)+ (M+	Abund %	Abund Sum %	79.07		81.71

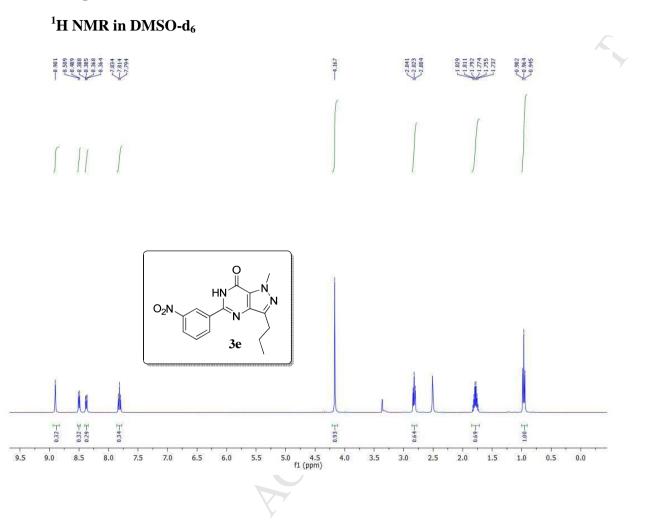
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4. Compound 3d



Sample Type Instrument M Acq Method IRM Calibrat Comment Sample Grou	Name tion Status	Samp Instru DAIL	R-OO.d ile iment 1 Y MS DESI.i	.m	Sample Nar Position User Name Acquired Ti	51 ime 2/23	3/2012	2 3:51:40 PM		MeO	HN HN N Br 3d
Compound	Table									L	
Compou	ind Label	RT	Mass		Formula		MF	G Formula		IFG Diff (ppm)	DB Formula
	5 H17 Br N4 O2	0.252	376.052	237	C16 H17 Br N4 O	2		H17 Br N4 O2		2.98	C16 H17 Br N4 O2
Compound	Label	m/z	R	RT	Algorithm		Mas	s			
Cpd 15: C16 O2		377.0596).252	Find by Molecula	ar Feature		Statement and a statement of the stateme			
1.4 1.2 1 0.8 0.6			377.05963 C16 H18 Br N4 O2					77.09222 4 Br2 N8 Na O4			
1.2 1 0.8 0.6 0.4 0.2 0 15	0 200 250 m Peak List) 300 :	350 400		500 550 60 vs. Mass-to-Chai	00 650 rge (m/z)	700	052 008 032 H34 Br2 N8 Na O4 032 H34 Br2 N8 Na O4	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectrum m/z	m Peak List z Abund	Form	350 400 C	Counts			700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectru <i>m/z</i> 377.05963	m Peak List z Abund 1 1696	Form 94.6 C16 H	350 400 C ula 118 Br N4 C	Counts v	vs. Mass-to-Char Ion (M+H)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectru <i>m/z</i> 377.05963 378.06236	m Peak List z Abund 1 1696 1 273	Form 94.6 C16 H 09.6 C16 H	350 400 C ula 118 Br N4 C 118 Br N4 C	D2	Vs. Mass-to-Chai Ion (M+H)+ (M+H)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778	z Abund 1 1696 1 273 1 1631	Form 94.6 C16 H 09.6 C16 H 18.4 C16 H	ula 118 Br N4 C 118 Br N4 C 118 Br N4 C	D2 D2 D2 D2	Ion (M+H)+ (M+H)+ (M+H)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041	z Abund 1 1696 1 273 1 1631 1 286	Form 94.6 C16 H 109.6 C16 H 18.4 C16 H 52.1 C16 H	ula 118 Br N4 C 118 Br N4 C 118 Br N4 C 118 Br N4 C 118 Br N4 C	22 22 22 22 22 22	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum <i>m/z</i> 377.05963 378.06236 378.06236 379.05778 380.06041 381.0631	z Abund 1 1696 1 273 1 1631 1 286 1 30	Form 94.6 C16 H 109.6 C16 H 18.4 C16 H 552.1 C16 H 73.8 C16 H	ula 118 Br N4 C 118 Br N4 C	202 02 02 02 02 02 02	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 381.0631 399.04091	z Abund 1 1696 1 273 1 1631 1 286 1 30 1 45	Form 94.6 C16 H 109.6 C16 H 18.4 C16 H 52.1 C16 H 73.8 C16 H 08.3 C16 H	ula 118 Br N4 C 118 Br N4 C	02 02 02 02 02 02 02 02 02 02 02 02 02	vs. Mass-to-Chai (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum <i>m/z</i> 377.05963 378.06236 378.06236 379.05778 380.06041 381.0631	z Abund 1 1696 1 273 1 1631 1 286 1 300 1 45 1 44	Form 94.6 C16 H 109.6 C16 H 18.4 C16 H 552.1 C16 H 73.8 C16 H	ula 118 Br N4 C 118 Br N4 C 117 Br N4 N 117 Br N4 N	02 02 02 02 02 02 02 02 02 02 02 02 02 0	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 381.0631 399.04091 401.03953	z Abund 1 1696 1 273 1 1631 1 286 1 300 1 45 1 44 1 122	Form 94.6 C16 H 09.6 C16 H 18.4 C16 H 52.1 C16 H 73.8 C16 H 08.3 C16 H 69.6 C16 H	ula 118 Br N4 C 118 Br N4 C 117 Br N4 N 117 Br N4 N 134 Br2 N8	202 202 202 202 202 202 202 202 202 202	vs. Mass-to-Chai (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 381.0631 399.04091 401.03953 775.09143	z Abund 1 1696 1 273 1 1631 1 286 1 30 1 44 1 122 1 122	Form 94.6 C16 H 109.6 C16 H 18.4 C16 H 552.1 C16 H 73.8 C16 H 108.3 C16 H 69.6 C16 H 53.9 C32 H	uia uia 118 Br N4 Q 118 Br N4 Q 117 Br N4 N 117 Br N4 N 117 Br N4 N 117 Br N4 N 117 Br N4 N	D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D	vs. Mass-to-Chai (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (2M+Na)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum m/z 377.05963 378.06236 379.05778 380.06041 381.0631 399.04091 401.03953 775.09143 777.09222 779.08826 Predicted Is	z Abund 1 1696 1 273 1 1631 1 286 1 30 1 44 1 122 1 122	Form 994.6 C16 H 109.6 C16 H 18.4 C16 H 52.1 C16 H 08.3 C16 H 08.3 C16 H 69.6 C16 H 53.9 C32 H 2646 C32 H	uia uia 118 Br N4 Q 118 Br N4 Q 117 Br N4 N 117 Br N4 N 117 Br N4 N 117 Br N4 N 117 Br N4 N	D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D	vs. Mass-to-Chai Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (2M+Na)+ (2M+Na)+		700	the second	850	900	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 381.0631 399.04091 401.03953 775.09143 777.09222 779.08826 Predicted Is Isotope	z Abund 1 1696 1 273 1 1631 1 286 1 300 1 45 1 44 1 122 1 14 Sotope Matcl m/z	Form 94.6 C16 09.6 C16 18.4 C16 73.8 C16 73.8 C16 69.6 C16 53.9 C32 2646 C32 61.1 C32 h Table Calc m/z	ula ula 118 Br N4 C 118 Br N4 C 117 Br N4 N 117 Br N4 N 134 Br2 N8 34 Br2 N8 34 Br2 N8 Diff (pr	Counts v D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2	vs. Mass-to-Chai Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (2M		/0	the second		Calc Abunc	
1.2 1 0.8 0.6 0.4 0.2 0 150 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 381.0631 399.04091 401.03953 775.09143 777.09226 Predicted IS Isotope 1	z Abund 1 1696 1 273 1 1631 1 286 1 300 1 45 1 44 1 12 1 14 sotope Matcl 377.05963	Form 94.6 C16 H 009.6 C16 H 18.4 C16 H 52.1 C16 H 73.8 C16 H 69.6 C16 H 69.6 C16 H 53.9 C22 H 2646 C32 H 61.1 C32 H Calc m/z 377.060	ula ula 118 Br N4 C 118 Br N4 C 117 Br N4 N 117 Br N4 N 134 Br2 N8 34 Br2 N8 34 Br2 N8 Diff (p) 077	Counts v 22 22 22 22 22 22 22 22 22 2	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (2M+Na)+(2M+Na)+ (2M+Na)+(2M+Na	rge (m/z)	100	750 800	43.26	Calc Abunc	41.77
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 380.06041 380.06041 399.04091 401.03953 775.09143 775.09143 777.09222 779.08826 Predicted Is Isotope 1 2	z Abund 1 1696 1 273 1 1631 1 286 1 300 1 45 1 44 1 12 1 44 1 12 1 500pe Matco 377.05963 378.06236 378.06236	Form 994.6 C16 H 009.6 C16 H 18.4 C16 H 52.1 C16 H 73.8 C16 H 69.6 C16 H 53.9 C32 H 2646 C32 H 61.1 C32 H Table Table Calc m/z 377.06(378.062 C38.062	ula 118 Br N4 Q 118 Br N4 Q 119 Br N4 N 117 Br N4 N 117 Br N4 N 117 Br N4 N 117 Br N4 N 118 Br N4 Q 118 Br N4 Q	Counts v D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2	vs. Mass-to-Chai (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (2M+Na)+	rge (m/z)	% 1 100 9.05	750 800	43.26 6.96	Calc Abunc	41.77 7.96
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 381.0631 399.04091 401.03953 775.09143 777.09222 779.08826 Predicted Is Isotope 1 2 3 3 3 3 3 3 3 3 3 3 3 3 3	Peak List z Abund 1 1696 1 273 1 1631 1 286 1 300 1 44 1 122 1 14 Sotope Matcl 377.05963 378.06236 379.05778	Form 994.6 C16 H 009.6 C16 H 18.4 C16 H 52.1 C16 H 52.1 C16 H 53.3 C16 H 53.9 C32 H 2646 C32 H 61.1 C32 H Calc m/z 377.06(377.050 379.058	350 400 ula	Counts v Co Co Co Co Co Co Co Co Co Co	VS. Mass-to-Chai Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (2M	Ic Abund %	% 100 9.05 9.41	750 800	43.26 6.96 41.58	Calc Abunc	41.77 7.96 41.52
1.2 1 0.8 0.6 0.4 0.2 0 15 MS Spectrum <i>m/z</i> 377.05963 378.06236 379.05778 380.06041 380.06041 380.06041 399.04091 401.03953 775.09143 775.09143 777.09222 779.08826 Predicted Is Isotope 1 2	z Abund 1 1696 1 273 1 1631 1 286 1 300 1 45 1 44 1 12 1 44 1 12 1 500pe Matco 377.05963 378.06236 378.06236	Form 994.6 C16 H 009.6 C16 H 18.4 C16 H 52.1 C16 H 73.8 C16 H 69.6 C16 H 53.9 C32 H 2646 C32 H 61.1 C32 H Table Table Calc m/z 377.060 378.062 378.062	350 400 ula	Counts v D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2	vs. Mass-to-Chai (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (2M+Na)+	rge (m/z)	% 1 100 9.05	750 800	43.26 6.96	Calc Abunc	41.77 7.96

5. Compound 3e

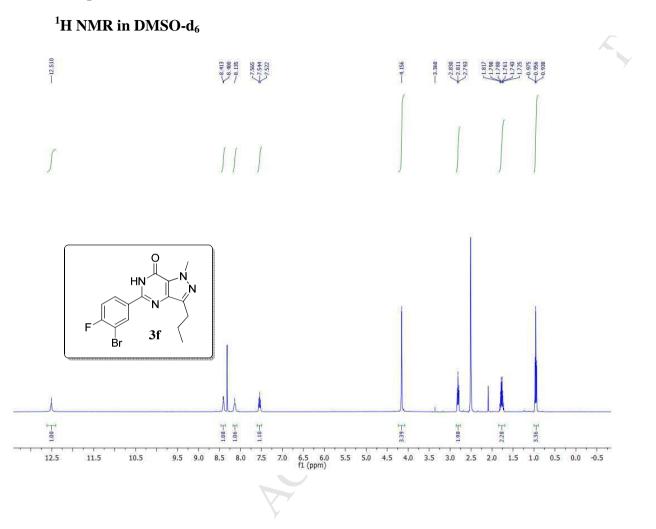


Qualitative Compound Report

Instrument M Acq Method IRM Calibrati Comment Sample Grou	ion Status	Success	ent 1 4S_25072012.m	Sample I Position User Nar Acquired DA Meth	Vial 29 me I Time 11/19/20	12 3:47:53 PM		O ₂ N	
Compound	Table	1.1.1							
Common	nd Label	RT	Mass	Formula		FG Formula		FG Diff (ppm)	DB Formula
	C15 H15 N5 O3		313.11702	C15 H15 N5		15 H15 N5 O3		1.49	C15 H15 N5 O3
MFE MS Spectr x10 ⁵ Cpc 2-		314.12430 55 5 H16 N5 O3 +	MFE Spectrun	n (0.136-0.530	min) GLR-01.d				
1.5- · 1-		314.1 C15 H1							
0.5	150 200 2 m Peak List	50 300 3	50 400 450 Counts	500 550 6 vs. Mass-to-C	00 650 700 7 harge (m/z)	50 800 850	900	950	
1 0.5 0 MS Spectru	150 200 2 m Peak List z Abund	50 300 3	Counts		500 650 700 7 Sharge (m/z)	50 800 850	900	950	
0.5	m Peak List z Abund	50 300 3	Counts	vs. Mass-to-C	00 650 700 7 harge (m/z)	50 800 850	900	950	
1 0.5 0 MS Spectru <i>m/z</i> 314.1243	m Peak List z Abund 1 2195	50 300 3 Formu 505.2 C15 H1	Counts la 6 N5 03	vs. Mass-to-C	00 650 700 7 harge (m/z)	50 800 850	900	950	
1 0.5 0 MS Spectru <i>m/z</i> <u>314.1243</u> 315.12725	z Abund 1 2195 1 391	50 300 3 Formu 505.2 C15 H1 (15.9 C15 H1	Counts la 6 N5 03 6 N5 03	vs. Mass-to-C Ion (M+H)+ (M+H)+	500 650 700 7 Charge (m/z)	50 800 850	900	950	
1 0.5 0 <i>m/z</i> 314.1243 315.12725 316.12967	m Peak List z Abund 1 2195 1 391 1 51	50 300 3 Formu 505.2 C15 H1 15.9 C15 H1 30.5 C15 H1	Counts a 6 N5 03 6 N5 03 6 N5 03	vs. Mass-to-C Ion (M+H)+ (M+H)+ (M+H)+	500 650 700 7 Charge (m/z)	50 800 850	900	950	
MS Spectru m/z 314.1243 315.12725 316.12967 336.10699	z Abund 1 2195 1 391 1 51 1 51 1 8	50 300 3 Formu 505.2 C15 H1 115.9 C15 H1 130.5 C15 H1 229.7 C15 H1	Counts a 6 N5 03 6 N5 03 6 N5 03	vs. Mass-to-C Ion (M+H)+ (M+H)+	500 650 700 7 Sharge (m/z)	750 800 850	900	950	
MS Spectru m/z 314.1243 315.12725 316.12967 336.10699 Predicted Is	z Abund 1 2195 1 391 1 51 1 6 sotope Matc	50 300 3 Formu 505.2 C15 H1 15.9 C15 H1 30.5 C15 H1 30.5 C15 H1 15.9 C15 H1 h Table	Counts 6 N5 O3 6 N5 O3 6 N5 O3 6 N5 O3 5 N5 Na O3	vs. Mass-to-C Ion (M+H)+ (M+H)+ (M+H)+ (M+Na)+	Charge (m/z)		900		1 Sum %
MS Spectru m/z 314.1243 315.12725 316.12967 336.10699	z Abund 1 2195 1 391 1 51 1 51 1 8 sotope Matc m/z	50 300 3 Formu 50.2 C15 H1 15.9 C15 H1 30.5 C15 H1 30.5 C15 H1 129.7 C15 H1 h Table Calc m/z	Counts 6 N5 03 6 N5 03 6 N5 03 6 N5 03 5 N5 Na 03 Diff (ppm)	vs. Mass-to-C Ion (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund %	Calc Abund %	Abund Sum %		950 Calc Abunc	
MS Spectru m/z 314.1243 315.12725 316.12967 336.10699 Predicted Is	z Abund 1 2195 1 391 1 51 1 8 sotope Matc m/z 314.1243 314.1243	Formu 50.2 C15 H1 15.9 C15 H1 30.5 C15 H1 30.5 C15 H1 22.7 C15 H1 Table Calc m/z 314.1247	Counts 6 N5 03 6 N5 03 6 N5 03 5 N5 Na 03 Diff (ppm) 7 1.49	vs. Mass-to-C Ion (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund % 2 100	Charge (m/z)	Abund Sum %	900 83.22 14.83		I Sum % 82.95 15.22

---- End Of Report ----

6. Compound 3f

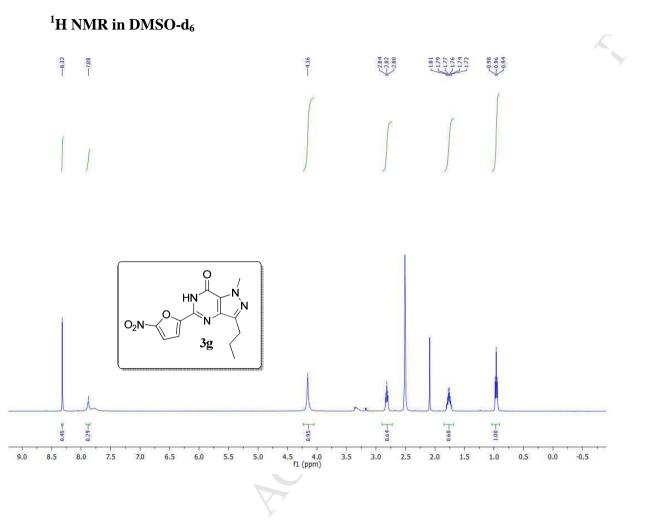


Qualitative Compound Report

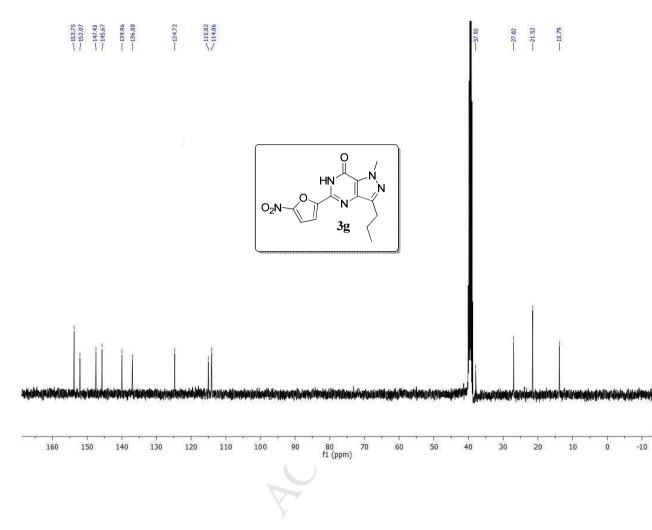
IRM Calibrati Comment Sample Group Compound	p Info	Success	S DESI.m	Sample Nam Position User Name Acquired Tim DA Method	2	2 12:20:28 PM	F	
							MFG Diff	
Compour	nd Label H14 Br F N4 O	RT 0.272	Mass 364.0336	Formula C15 H14 Br F N4 O		FG Formula H14 Br F N4 O	(ppm) -0.26	DB Formula C15 H14 Br F N4 O
Cpd 20. C15		0.272	304.0330	C13 H14 BI F N4 U			-0.26	C13 H14 BI F N4 U
Compound I	ahel	m/z	RT	Algorithm	Ма	22		
Cpd 20: C15 I		365.04088	0.272	Find by Molecular		Contract to a citize di su cont		
0	THE DIT NOT	303.04000	0.272	ind by molecular	Catale 304	.0330		
			0.15 H15 Br F N4 0					
0.2	150 200 2	50 300 35				50 800 850 900	950	
0		50 300 35	60 400 450	500 550 600 vs. Mass-to-Charg		50 800 850 900	950	
0	m Peak List	50 300 35	60 400 450			50 800 850 900	950	
0 MS Spectrum <i>m/z</i> 365.04088	m Peak List z Abund		60 400 450 Counts	vs. Mass-to-Charg		50 800 850 900	950	
0 MS Spectrum <i>m/z</i> 365.04088 366.04398	z Abund 1 100778 1 15676	Formula C15 H15 Br F C15 H15 Br F	N4 O	vs. Mass-to-Charg Ion (M+H)+ (M+H)+		50 860 850 960	950	
0 MS Spectrum <i>m/z</i> 365.04088 366.04398 367.03895	z Abund 1 100778 1 15676 1 100156	Formula C15 H15 Br F C15 H15 Br F C15 H15 Br F	N4 0 N4 0 N4 0	vs. Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+		50 800 850 900	950	
0 MS Spectrue m/z 365.04088 366.04398 367.03895 368.04184	z Abund 1 100778 1 15676 1 100156 1 15596.4	Formula C15 H15 Br F C15 H15 Br F C15 H15 Br F C15 H15 Br F	N4 0 N4 0 N4 0 N4 0 N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+		50 800 850 900) 950	
0 MS Spectruu m/z 365.04088 366.04398 367.03895 368.04184 369.04489	Z Abund 1 100778 1 15676 1 100156 1 15596.4 1 1446.3	Formula C15 H15 Br F C15 H15 Br F C15 H15 Br F C15 H15 Br F C15 H15 Br F	N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+		50 860 850 960	0 950	
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258	z Abund 1 100778 1 15676 1 100156 1 15596.4 1 1446.3 1 3596.3	Formula C15 H15 Br F C15 H14 Br F	N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+		50 800 850 900	0 950	
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 388.02589	z Abund 1 100778 1 15576 1 100156 1 15596.4 1 1446.3 1 3596.3 1 789.5	Formula C15 H15 Br F C15 H14 Br F C15 H14 Br F	N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+		50 800 850 900	0 950	
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 388.02589 389.02051	Peak List z Abund 1 100778 1 15676 1 15596.4 1 154596.3 1 15596.3 1 3596.3 1 789.5 1 3716.2	Formula C15 H15 Br F C15 H14 Br F	N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+		50 800 850 900	0 950	
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 388.02589 389.02051 402.99514	Peak List z Abund 1 100778 1 155676 1 15596.4 1 15596.4 1 3596.3 1 3596.3 1 3789.5 1 3716.2 1 868	Formula C15 H15 Br F C15 H14 Br F C15 H14 Br F	N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+K)+		50 800 850 900	0 950	
MS Spectrum m/z 365.04088 366.04398 368.04184 369.04489 387.02258 388.02589 389.02051 402.99514 404.99219	Peak List z Abund 1 100778 1 15576 1 15596.4 1 1446.3 1 3596.3 1 3716.2 1 868 1 918	Formula C15 H15 Br F C15 H14 Br F C15 H14 Br F C15 H14 Br F	N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+		50 800 850 900	0 950	
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 388.02589 389.02051 402.99514	Peak List z Abund 1 100778 1 15576 1 15596.4 1 1446.3 1 3596.3 1 3716.2 1 868 1 918	Formula C15 H15 Br F C15 H14 Br F C15 H14 Br F C15 H14 Br F	N4 0 N4 0	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+K)+ (M+K)+		50 800 850 900	Calc Abunc	d Sum %
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 388.02589 389.02051 402.99514 402.99519 Predicted Is Isotope 1	z Abund 1 100778 1 15576 1 15596.4 1 1446.3 1 3596.3 1 3576.2 1 3716.2 1 918	Formula C15 H15 Br F C15 H14 Br F C15 H14 Br F C15 H14 Br F C15 H14 Br F	N4 0 N4 0 Diff (ppm)	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ Calc	ge (m/z) : Abund % 100	Abund Sum %		d Sum % 42.34
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 388.02589 389.02051 402.99514 404.99219 Predicted Is Isotope 1 1 2	Peak List z Abund 1 100778 1 15676 1 15596.4 1 15596.4 1 3596.3 1 3596.3 1 3716.2 1 3688 1 918 xotope Matcom/z 365.04088 365.04088 366.04398	Formula C15 H15 Br F C15 H14 Br F	N4 0 450 Counts Counts N4 0 N4 0 N4 0 N4 0 N4 Na 0 N4 Na 0 N4 Na 0 N4 Na 0 Diff (ppm) -0.25 5 -0.925	vs. Mass-to-Charg Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+K)+ (M+	; Abund % 100 17.9	Abund Sum % 43 6	Calc Abunc .11 .71	42.34 7.58
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 389.02551 402.99514 404.99219 Predicted Is Isotope 1 1 2 3 3 2 3 3 3 3 3 3 3 3 3 3 3 3 3	Peak List z Abund 1 100778 1 15576 1 15596.4 1 15596.4 1 1446.3 1 3596.3 1 3789.5 1 3716.2 1 918 otope Matc m/z 365.04088 367.03895	Formula C15 H15 Br F C15 H14 Br F C15 H14 Br F C15 H14 Br F C15 H14 Br F Calc m/z 366.04365 367.03886	N4 0 450 Counts Counts N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 Na 0 N4 Na 0 N4 Na 0 O Diff (ppm) 3 -0.28 -0.92 5 -0.24	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+K)+ (e (m/z) Abund % 100 17.9 98.99	Abund Sum % 43 6 42	Calc Abunc .11 .71 .85	42.34 7.58 41.91
MS Spectrum m/z 365.04088 366.04398 367.03895 368.04184 369.04489 387.02258 388.02589 389.02051 402.99514 404.99219 Predicted Is Isotope 1 1 2	Peak List z Abund 1 100778 1 15676 1 15596.4 1 15596.4 1 3596.3 1 3596.3 1 3716.2 1 3688 1 918 xotope Matcom/z 365.04088 365.04088 366.04398	Formula C15 H15 Br F C15 H14 Br F	N4 0 450 Counts Counts N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 0 N4 Na 0 N4 Na 0 Diff (ppm) 3 35 -0.28 5 -0.53	vs. Mass-to-Charg (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+K)+ (; Abund % 100 17.9	Abund Sum % 43 6 42 6	Calc Abunc .11 .71	42.34 7.58

--- End Of Report ---

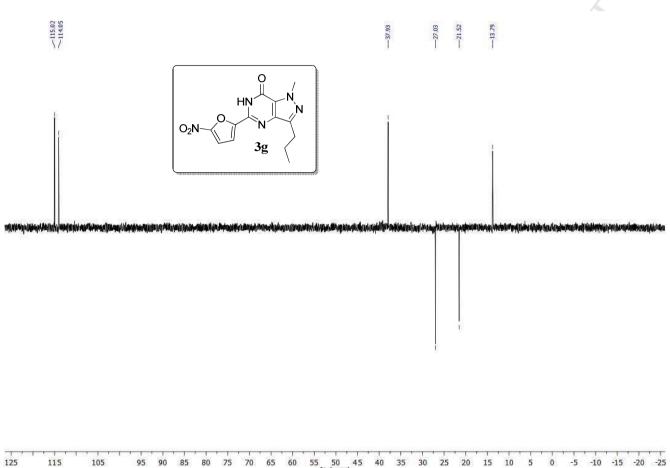
7. Compound 3g







DEPT NMR in DMSO-d₆



95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 f1 (ppm) 115 105

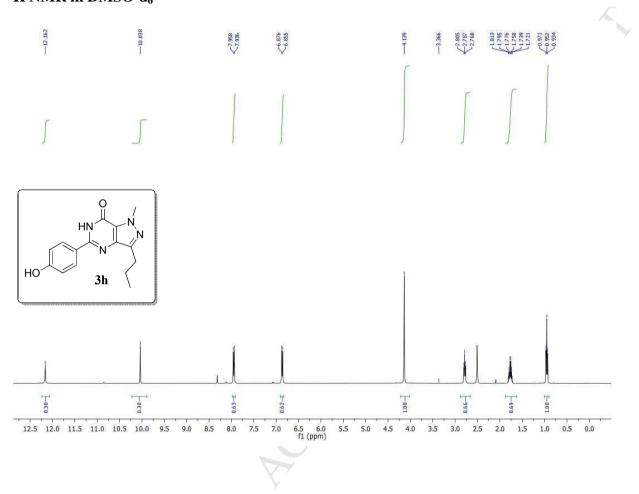
Qualitative Compound Report

Data File Sample Type Instrument M Acq Method IRM Calibrat Comment Sample Grou	Name ion Status	Success	ent 1 IS DESI.m	Sample N Position User Nam Acquired DA Metho	26 ne Time 1/18/	2012 11:41:39 AM	ļ	O₂N√	
Compound								FG Diff	
	Ind Label C13 H13 N5 O4	RT 0.236	Mass 303.09682	Formula C13 H13 N5 C	04	MFG Formula C13 H13 N5 O4	(-0.21	DB Formula C13 H13 N5 O4
Compound	Labol	m/7	RT	Algorithm		Mass			
Cpd 30: C13		<i>m/z</i> 304.10409	0.236	Find by Moleci		distant in the second se			
3 - 2.5 - 1.5 - 1 - 0.5 - 0 -	150 200 2	* 304.10409 * 304.10409 C13 H14 N5 O4		500 550 60		0 750 800 850	900 9	950	
			Counts	vs. Mass-to-Cl	harge (m/z)				
MS Spectru	m Peak List								
MS Spectru m/z	z Abund	Formula	a	Ion					
	z Abund	Formul		Ion (M+H)+	_				
m/z	z Abund 1 3520		N5 04						
m/z 304.10409 305.10722 306.10937	z Abund 1 3520 1 469 1 58	052.5 C13 H14 982.8 C13 H14 822.5 C13 H14	N5 04 N5 04 N5 04	(M+H)+ (M+H)+ (M+H)+					
m/z 304.10409 305.10722 306.10937 307.11129	z Abund 1 3520 1 469 1 58 1 58	052.5 C13 H14 982.8 C13 H14 922.5 C13 H14 595.1 C13 H14	N5 04 N5 04 N5 04 N5 04 N5 04	(M+H)+ (M+H)+ (M+H)+ (M+H)+					
<i>m/z</i> 304.10409 305.10722 306.10937 307.11129 326.08634	z Abund 1 3520 1 469 1 58 1 58 1 249 1 249	052.5 C13 H14 982.8 C13 H14 322.5 C13 H14 595.1 C13 H14 963.2 C13 H13	N5 04 N5 04 N5 04 N5 04 N5 04 N5 Na 04	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+					
m/z 304.10409 305.10722 306.10937 307.11129 326.08634 327.08911	z Abund 1 3520 1 469 1 58 1 58 1 249 1 38 1 38	052.5 C13 H14 982.8 C13 H14 822.5 C13 H14 595.1 C13 H14 963.2 C13 H13 812.6 C13 H13	N5 04 N5 04 N5 04 N5 04 N5 04 N5 Na 04 N5 Na 04	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+					
m/z 304.10409 305.10722 306.10937 307.11129 326.08634 327.08911 328.0897	z Abund 1 3520 1 469 1 56 1 56 1 249 1 38 1 38	052.5 C13 H14 082.8 C13 H14 032.5 C13 H14 0595.1 C13 H14 063.2 C13 H13 0312.6 C13 H13 044 C13 H13	N5 04 N5 04 N5 04 N5 04 N5 04 N5 Na 04 N5 Na 04	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+					
m/z 304.10409 305.10722 306.10937 307.11129 326.08634 327.08911 328.0897 Predicted Is	z Abund 1 3520 1 469 1 58 1 58 1 249 1 38 1 38 1 38 1 5000000000000000000000000000000000000	552.5 C13 H14 582.8 C13 H14 522.5 C13 H14 595.1 C13 H14 563.2 C13 H14 563.2 C13 H13 512.6 C13 H13 744 C13 H13 Table	N5 04 N5 04 N5 04 N5 04 N5 04 N5 Na 04 N5 Na 04 N5 Na 04	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+					
m/z 304.10409 305.10722 306.10937 307.11129 326.08634 327.08911 328.0897 Predicted Is Isotope	z Abund 1 352(1 469 1 58 1 51 1 249 1 38 1 38 1 38 1 38 1 38 1 38 1 38 1 38	52.5 C13 H14 282.8 C13 H14 322.5 C13 H14 595.1 C13 H14 663.2 C13 H14 312.6 C13 H13 744 C13 H13 ch Table Calc m/z	N5 04 N5 04 N5 04 N5 04 N5 04 N5 Na 04 N5 Na 04 N5 Na 04 Diff (ppm)	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ Abund %	Calc Abund %			Calc Abund	
m/z 304.10409 305.10722 306.10937 307.11129 326.08634 327.08911 328.0897 Predicted It Isotope 1	z Abund 1 3520 1 469 1 551 1 249 1 249 1 301 sotope Matc m/z 304.10409 304.10409	3052.5 C13 H14 3028.8 C13 H14 3022.5 C13 H14 3022.5 C13 H14 505.1 C13 H14 302.2 C13 H13 312.6 C13 H13 744 C13 H13 745 C13 H13 746 C13 H13 747 C13 H13 748 C13 H13 749 C13 H13 740 C13 H13 741 C13 H13 742 C13 H13 744 C14 H13 745 Galc m/z 304.10400 304.10400 M14	N5 04 N5 04 N5 04 N5 04 N5 Na 04 N5 Na 04 N5 Na 04 N5 Na 04 Diff (ppm) 3 -0.21	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ (M+Na)+	241.5.1.	100	86.83	Calc Abund	84.43
m/z 304.10409 305.10722 306.10937 307.11129 326.08634 327.08911 328.0897 Predicted It Isotope 1 2 2	z Abund 1 3520 1 469 1 56 1 249 1 249 1 31 1 30 1 304.10409 305.10722 305.10722	552.5 C13 H14 382.8 C13 H14 322.5 C13 H14 595.1 C13 H14 363.2 C13 H13 312.6 C13 H13 312.6 C13 H13 312.6 C13 H13 312.6 C13 H13 314.6 C13 H13 315.0 C13 H13 316.7 C14 H13 317.6 C14 H13 310.6 C13 H13 310.6 C14 H13 310.7 C14 H14 304.10400 305.10667	N5 04 N5 04 N5 04 N5 04 N5 Na 04 N5 Na 04 N5 Na 04 Diff (ppm) 3 -0.21 1 -1.66	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ Abund % 100 13.35		100 16.2	86.83 11.59	Calc Abund	84.43 13.68
m/z 304.10409 305.10722 306.10937 307.11129 326.08634 327.08911 328.0897 Predicted It Isotope 1	z Abund 1 3520 1 469 1 56 1 249 1 249 1 301 sotope Matc m/z 304.10409 305.10722 306.10937 306.10937	052.5 C13 H14 982.8 C13 H14 932.5 C13 H14 955.1 C13 H14 963.2 C13 H13 912.6 C13 H13 744 C13 H13 744 C13 H14 03.04.10400 304.10400 305.1067 306.1089	N5 04 N5 04 N5 04 N5 04 N5 Na 04 N5 Na 04 N5 Na 04 Diff (ppm) 3 -0.21 1 -1.66 1 -1.48	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ (M+Na)+ (M+Na)+ Abund % 100 13.35		100	86.83	Calc Abund	84.43

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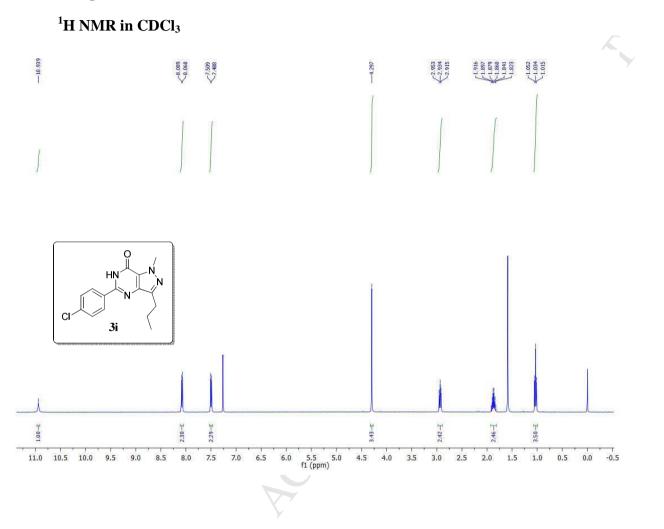
8. Compound 3h

¹H NMR in DMSO-d₆



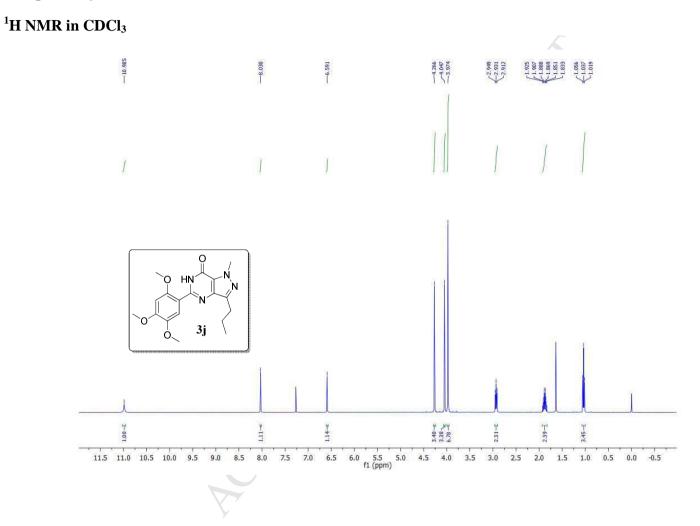
Sample Type			Sampl			Position	Vial 1	.9			O L N
Instrument N	ame			ment 1		User Nar					HŅ´ \
Acq Method				_MS_250	72012.m	Acquired	to the contract to the Real	9/2012 1:47:31 P	M		
IRM Calibrati	on Sta	tus	Succe	SS		DA Meth	od as.m			Í	N 1
Comment											
Sample Group	•	Info.								HO	° 3h {
Compound 1	Table		-						м	FG Diff	
Compour	nd Lab	el	RT	Mass		Formula		MFG Formula		(ppm)	DB Formula
Cpd 5: C			0.171	284.12	2654	C15 H16 N4	02	C15 H16 N4 02	2	2.75	C15 H16 N4 O2
Compound I Cpd 5: C15 H		02	285.1338	2	0.171	Find by Moleo	cular Feature	284.12654			
MFE MS Spectr	0	5 H16 M	V4 O2: +	MFE Sp	ectrum (0.126-0.405 r	nin) GLR-05.	9	-		
			285.13382 C15 H17 N4 O2								
1-			285.13382 5 H17 N4 (
0.8			H1							100	
0.6			21 C15								
0.01											
0.4											
										100	
0.4 -											
0.4 0.2 0		200 25 k List	50 300	350 4	00 450 Counts	500 550 vs. Mass-to-C	600 650 7 Charge (m/z)	00 750 800	850 900	950	
0.4 -	m Pea		50 300 Form		00 450 Counts	500 550 vs. Mass-to-C	600 650 7 Charge (m/z)	00 750 800	850 900	950	
0.4 0.2 0 MS Spectru	m Pea	k List		ula	Counts	vs. Mass-to-C	600 650 7 Charge (m/z)	00 750 800	850 900	950	
0.4 0.2 0 MS Spectru	m Pea z A	bund 1226	Form	ula 117 N4 O	Counts 2	vs. Mass-to-C	600 650 7 Charge (m/z)	00 750 800	850 900	950	
0.4 0.2 0 MS Spectru <i>m/z</i> 285.13382	m Pea z A 1	k List bund 1226: 233	Form	117 N4 0	Counts	Ion (M+H)+	600 650 7 harge (m/z)	00 750 800	850 900	950	
0.4 - 0.2 - 0 - MS Spectru <i>m/z</i> 285.13382 286.13654	m Pea z A 1 1 1	ak List bund 1226: 233: 310	Form 15.1 C15 H 72.1 C15 H 55.2 C15 H	117 N4 0	Counts	Vs. Mass-to-C Ion (M+H)+ (M+H)+ (M+H)+	Charge (m/z)				
0.4 0.2 0 <i>m/z</i> 285.13382 286.13654 287.13921	m Pea z A 1 1 1 sotopo m/z	hk List bund 1226: 233: 310 e Match	Form 15.1 C15 H 72.1 C15 H 55.2 C15 H 1 Table Calc m/z	117 N4 0 117 N4 0 117 N4 0 117 N4 0	Counts	Vs. Mass-to-C Ion (M+H)+ (M+H)+ (M+H)+ Abund %	Calc Abund °	% Abund Sur	m %	Calc Abund	
0.4 0.2 0 MS Spectru <i>m/z</i> 285.13382 286.13654 287.13921 Predicted Is	m Pea z A 1 1 1 sotopo m/z	bund 1226: 233: 310 e Match	Form 15.1 C15 F 72.1 C15 F 55.2 C15 F 1 Table	117 N4 0 117 N4 0 117 N4 0 117 N4 0 117 N4 0	Counts	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ Abund % 100	Charge (m/z)			Calc Abune	1 Sum % 83.41 14.98

9. Compound 3i

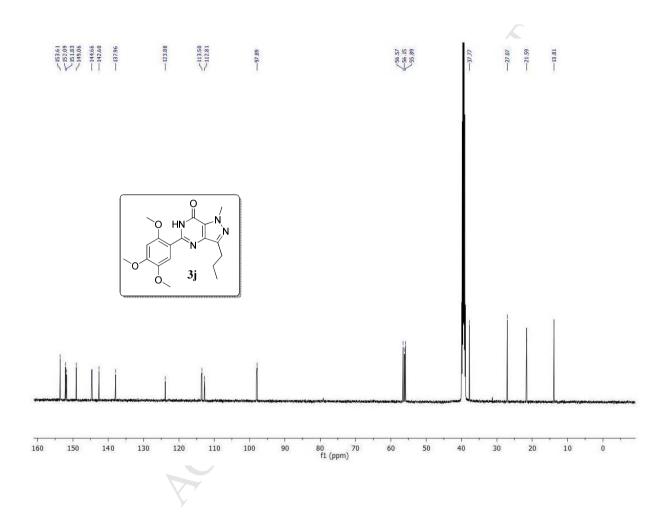


Cpd 8: C15 H15 Cl N4 0 0.172 302.09264 C15 H15 Cl N4 0 C15 H15 Cl N4 0 2.64 C15 H15 Compound Label m/z RT Algorithm Mass 302.09264 Cpd 8: C15 H15 Cl N4 0 303.09991 0.172 Find by Molecular Feature 302.09264 MFE MS Spectrum Cpd 8: C15 H15 Cl N4 0: + MFE Spectrum (0.124-0.484 min) GLR-09.d Mass 302.09264 1.75 MEE MS Spectrum x10 5 Cpd 8: C15 H15 Cl N4 0: + MFE Spectrum (0.124-0.484 min) GLR-09.d Mass 0.75 0.75 0.75 0.75 0.75	ample Group Info.						3i
Compound Label RT Mass Formula MFG Formula MFG Diff DB For Cpd 8: C15 H15 Cl N4 0 0.172 302.09264 C15 H15 Cl N4 0 C15 H15 Cl N4 0 2.64 C15 H15 Compound Label m/z RT Algorithm Mass Mass Cpd 8: C15 H15 Cl N4 0 303.09991 0.172 Find by Molecular Feature 302.09264 MFE MS Spectrum Cpd 8: C15 H15 Cl N4 0: + MFE Spectrum (0.124-0.484 min) GLR-09.d Mass 302.09264 1.75						<u></u>	
Cpd 8: C15 H15 Cl N4 0 0.172 302.09264 C15 H15 Cl N4 0 C15 H15 Cl N4 0 2.64 C15 H15 Compound Label m/z RT Algorithm Mass 302.09264 Cpd 8: C15 H15 Cl N4 0 303.09991 0.172 Find by Molecular Feature 302.09264 MFE MS Spectrum Cpd 8: C15 H15 Cl N4 0: + MFE Spectrum (0.124-0.484 min) GLR-09.d Mass 302.09264 1.75 Mass 1.75 Mass 0.75							
Cpd 8: C15 H15 Cl N4 0 303.09991 0.172 Find by Molecular Feature 302.09264 MFE MS Spectrum x10 5 Cpd 8: C15 H15 Cl N4 O: + MFE Spectrum (0.124-0.484 min) GLR-09.d 1.75 1.75							DB Formula C15 H15 Cl N4
Cpd 8: C15 H15 Cl N4 O 303.09991 0.172 Find by Molecular Feature 302.09264 MFE MS Spectrum x10 5 Cpd 8: C15 H15 Cl N4 O: + MFE Spectrum (0.124-0.484 min) GLR-09.d 1.75 1.75							
x10 s Cpd 8: C15 H15 Cl N4 O: + MFE Spectrum (0.124-0.484 min) GLR-09.d 1.75 1.25 1.25 0.75 0.75 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 Counts vs. Mass-to-Charge (m/z) MS Spectrum Peak List m/z z M/2 I 34317.9 (15 H16 Cl N4 0 (M+H)+ 306.10056 1 1 12004 Cl 5 H16 Cl N4 0 (M+H)+ 307.101 1 1 1500pe M/z Calc M/z Calc M/z Diff (ppm) Abund % Abund % Abund % Calc Abund Sum % Calc Abund Sum %		and the second se	a second s		control to and the second second second second		
x10 s Cpd 8: C15 H15 Cl N4 0: + MFE Spectrum (0.124-0.484 min) GLR-09.d 1.75 1.75 1.25 0.75 1.50 2.00 2.50 3.00 3.50 5.50 6.00 6.50 7.00 7.50 8.00							
1.75 1.75 1.25 1.25 1.25 1.25 0.75 0.5 0.75 0.5 0.25 150 200 250 300 350 400 450 500 650 700 750 800 850 MS Spectrum Peak List m/z z Abund Formula Ion (M+H)+ 303.09991 1 194356 C15 H16 Cl N4 0 (M+H)+ 306.10056 1 12004 C15 H16 Cl N4 0 (M+H)+ 306.10056 1 12004 C15 H16 Cl N4 0 (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 0 (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 0 (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 0 (M+H)+ 307.101 1 1578.7 Cl M 0 (M+H)+ 307.101 1 1578.7 Cl M 0 (M+H)+ 303.09991 303.10072 2.65 100 100 64.41 63		AFE Spectrum	n (0.124-0.484	4 min) GLR-09.d			
0.75 0.25							
0.75 0.25 0.25 0.25 0.25 0.25 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 Counts vs. Mass-to-Charge (m/z) MS Spectrum Peak List m/z z Abund Formula Ion 303.09991 1 194356 C15 H16 Cl N4 0 (M+H)+ 304.10298 1 34317.9 C15 H16 Cl N4 0 (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 0 (M+H)+ 305.09761 1 12004 C15 H16 Cl N4 0 (M+H)+ 305.0056 1 12004 C15 H16 Cl N4 0 (M+H)+ Predicted Isotope Match Table Isotope m/z Calc m/z Diff (ppm) Abund % Calc Abund % Abund Sum % Calc Abund Sum %	1.5						
0.75 0.25 0.25 0.25 0.25 0.25 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 Counts vs. Mass-to-Charge (m/z) MS Spectrum Peak List m/z z Abund Formula Ion 303.09991 1 194356 C15 H16 Cl N4 0 (M+H)+ 304.10298 1 34317.9 C15 H16 Cl N4 0 (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 0 (M+H)+ 305.09761 1 12004 C15 H16 Cl N4 0 (M+H)+ 305.0056 1 12004 C15 H16 Cl N4 0 (M+H)+ Predicted Isotope Match Table Isotope m/z Calc m/z Diff (ppm) Abund % Calc Abund % Abund Sum % Calc Abund Sum %	1.25						
0.75 0.25	1 000						
0.5 0.25 0 250 300 350 400 450 500 650 700 750 800 850 MS Spectrum Peak List m/z z Abund Formula Ion 100 1 194356 C15 H16 Cl N4 0 (M+H)+ 303.09991 1 194356 C15 H16 Cl N4 0 (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 0 (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 0 (M+H)+ MHH)+ 305.09761 1 1044.7 C15 H16 Cl N4 0 (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 0 (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 0 (M+H)+ Predicted Isotope Match Table Matching Calc Abund % Abund % Calc Abund Sum % Cal	0.75						
m/z z Abund Formula Ion 303.09991 1 194356 C15 H16 Cl N4 O (M+H)+ 304.10298 1 34317.9 C15 H16 Cl N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ Predicted Isotope Match Table Ioff (ppm) Abund % Calc Abund % Abund Sum % Calc Abund Sum % 1 303.09991 303.10072 2.65 100 100 64.41 63	0.5						
m/z z Abund Formula Ion 303.09991 1 194356 C15 H16 Cl N4 O (M+H)+ 304.10298 1 34317.9 C15 H16 Cl N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 305.09761 1 159485.7 C15 H16 Cl N4 O (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ Botope m/z Calc M2 MH)+ Calc Abund % Abund Sum % Calc Abund Sum % 1 303.09991 303.10072 2.65 100 100 64.41 62	0.25						
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m/z z Abund Formula Ion 303.09991 1 194356 C15 H16 CI N4 O (M+H)+ 304.10298 1 34317.9 C15 H16 CI N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 CI N4 O (M+H)+ 305.09761 1 59485.7 C15 H16 CI N4 O (M+H)+ 306.10056 1 12004 C15 H16 CI N4 O (M+H)+ 307.101 1 1578.7 C15 H16 CI N4 O (M+H)+ Predicted Isotope Match Table (M+H)+ (M+H)+ Isotope m/z Calc m/z Diff (ppm) Abund % Calc Abund Sum % Calc Abund Sum % 1 303.09991 303.10072 2.65 100 100 64.41 63	S Spectrum Peak List	Counts	vs. 101055-10-0	sharge (m/z)			
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305.09761 1 59485.7 C15 H16 Cl N4 O (M+H)+ 306.10056 1 12004 C15 H16 Cl N4 O (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ Predicted Isotope Match Table Isotope M/z Calc m/z Diff (ppm) Abund % Calc Abund Sum % Calc Abund Sum % 1 303.09991 303.10072 2.65 100 100 64.41 62		40					
306.10056 1 12004 C15 H16 Cl N4 O (M+H)+ 307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ Predicted Isotope Match Table Abund % Calc Abund % Abund Sum % Calc Abund Sum % 1 303.09991 303.10072 2.65 100 100 64.41 62							
307.101 1 1578.7 C15 H16 Cl N4 O (M+H)+ Predicted Isotope Match Table Isotope m/z Calc m/z Diff (ppm) Abund % Calc Abund % Abund Sum % Calc Abund Sum % 1 303.09991 303.10072 2.65 100 100 64.41 62							
Intersection of the section of							
Isotope m/z Calc m/z Diff (ppm) Abund % Calc Abund % Abund Sum % Calc Abund Sum % 1 303.09991 303.10072 2.65 100 100 64.41 65		+0	(M+H)+				
1 303.09991 303.10072 2.65 100 100 64.41 65		Diff (ppm)	Abund %	Calc Abund %	Abund Sum %	Calc Abund	Sum %
							63.28
	2 304.10298 304.10359						11.33
							21.33
			-				3.7
5 307.101 307.10338 7.75 0.81 0.56 0.52 0	5 307.101 307.10338	7.75	0.81	0.56	0.5	52	0.35

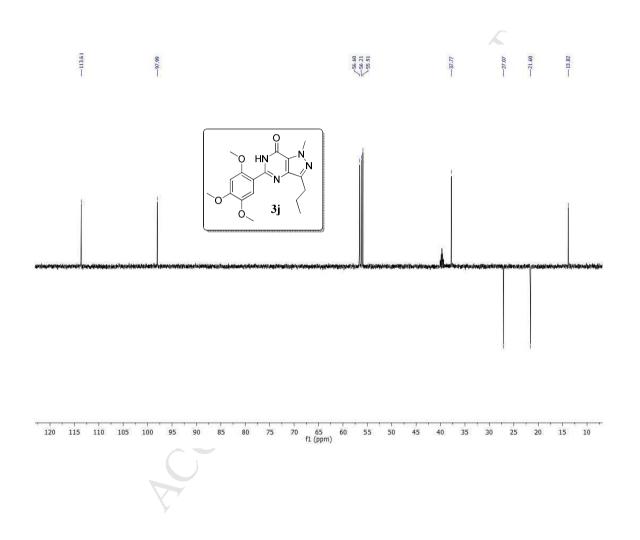
10. Compound 3j



¹³C NMR in DMSO-d₆



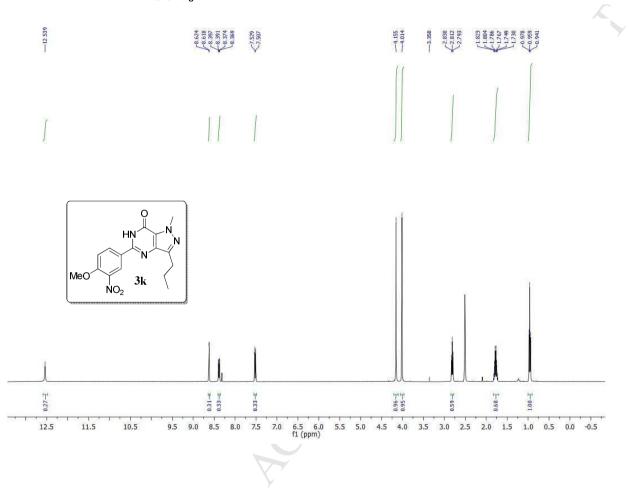
DEPT NMR in DMSO-d₆



Sample Type Instrument N Acq Method IRM Calibrati Comment			GLR-10.d Sample Instrume vishal_M Success		Sample N Position User Nam Acquired DA Metho	Vial 23 ne Time 11/19/201	2 2:08:52 PM			
Sample Group	•	Info.						- (<
Compound 1	Tabl	e					in the second second	1 1/2	GDiff	
Composi		hal	RT	Mass	Formula	ME	G Formula		opm)	DB Formul
Compou		22 N4 O4		358.16411	C18 H22 N4 (8 H22 N4 O4		0	C18 H22 N4
сри в. с	10 11	22 114 04	0.172	550.10111	CAUTILETT					
Compound	ahe		m/z	RT	Algorithm	Mas	s			
Cpd 8: C18 H			359.17139	0.172	and the second se	ular Feature 358.	The second state of the se			
Cpu 0. 010 11				0.1.1		New Addition 1993				
0.8 0.6 0.4 0.2 0	150	200 25	50 300 35	C18 H23 N4 04 397.12598 C18 H23 N4 04 397.12598 C18 H23 N4 04	500 550 6 vs. Mass-to-C	00 650 700 7	20 20 20 20 20 20 20 20 20 20 20 20 20 2	900 9	50	
MS Spectru	_		1		Ion					
m/z	z	Abund	Formul		(M+H)+					
359.17139 360.17386			05.2 C18 H23	and the second se	(M+H)+					
			58.8 C18 H23		(M+H)+					
361 17604			41.5 C18 H23		(M+H)+					
361.17604 362.17862	1			2 N4 Na O4	(M+Na)+					
361.17604 362.17862 381.15182	-	216	37.3 C18 H22		(M+Na)+					
362.17862	1		37.3 C18 H22 38.3 C18 H22	2 N4 Na O4	(M+Na)+					
362.17862 381.15182	1	32		2 N4 Na O4	(M+K)+					
362.17862 381.15182 382.15722	1 1 1	32	38.3 C18 H22							
362.17862 381.15182 382.15722 397.12598	1 1 1	32 13 358	38.3 C18 H22 78.5	1 N8 Na O8	(M+K)+ (2M+Na)- (2M+Na)-	+				
362.17862 381.15182 382.15722 397.12598 739.31527 740.31824 741.3231	1 1 1 1 1 1	32: 13 358 145 34	38.3 C18 H22 78.5 95.2 C36 H44 22.2 C36 H44 11.4 C36 H44	4 N8 Na O8 4 N8 Na O8	(M+K)+ (2M+Na)-	+				
362.17862 381.15182 382.15722 397.12598 739.31527 740.31824 741.3231 Predicted I	1 1 1 1 1 50t0	32 13 358 145 34 pe Matc	38.3 C18 H22 78.5 95.2 95.2 C36 H44 22.2 C36 H44 11.4 C36 H44 Table	4 N8 Na O8 4 N8 Na O8 4 N8 Na O8	(M+K)+ (2M+Na)- (2M+Na)- (2M+Na)-	+ + + +			Cala Ab	d Sum %
362.17862 381.15182 382.15722 397.12598 739.31527 740.31824 741.3231 Predicted I Isotope	1 1 1 1 1 soto	32: 13 358 145 34 pe Matcl z	38.3 C18 H22 78.5	4 N8 Na O8 4 N8 Na O8 4 N8 Na O8 Diff (ppm)	(M+K)+ (2M+Na)- (2M+Na)- (2M+Na)- (2M+Na)-	+ + + Calc Abund %	Abund Sum %	92.20	Calc Abune	
362.17862 381.15182 382.15722 397.12598 739.31527 740.31824 741.3231 Predicted I Isotope	1 1 1 1 1 50tc	32: 13 358 145 34 9 pe Matcl z 359.17139	38.3 C18 H22 78.5 95.2 C36 H44 22.2 C36 H44 11.4 C36 H44 h Table Calc m/z 359.1713	 N8 Na O8 N8 Na O8 N8 Na O8 Diff (ppm) -0.01 	(M+K)+ (2M+Na)- (2M+Na)- (2M+Na)- (2M+Na)- Abund % 100	+ + + Calc Abund % 100		82.36	Calc Abune	80.22
362.17862 381.15182 382.15722 397.12598 739.31527 740.31824 741.3231 Predicted I Isotope	1 1 1 1 soto	32: 13 358 145 34 pe Matcl z	38.3 C18 H22 78.5	 N8 Na O8 N8 Na O8 N8 Na O8 Diff (ppm) 8 -0.01 5 1.35 	(M+K)+ (2M+Na)- (2M+N	+ + + Calc Abund % 100 21.35		82.36 15.39 2.03	Calc Abund	

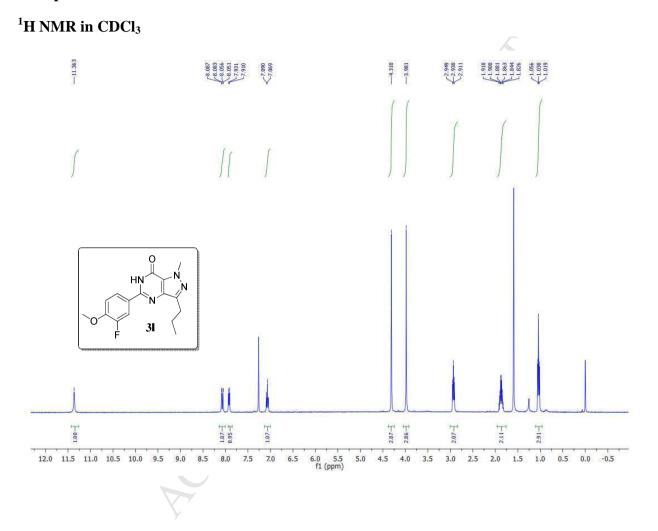
11. Compound 3k



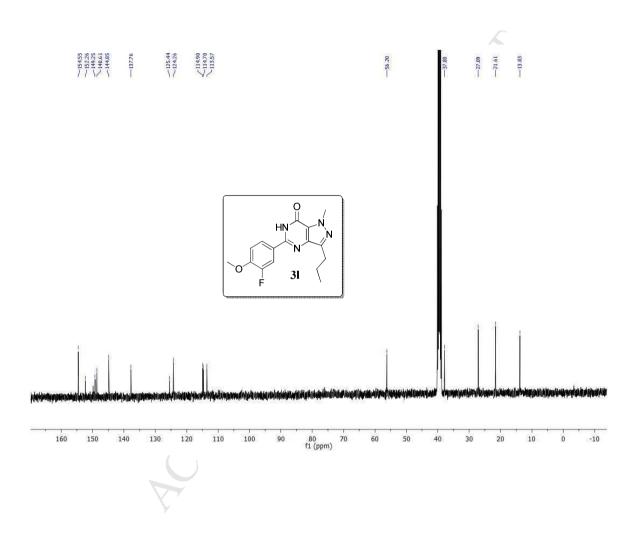


Sample Type Instrument M Acq Method IRM Calibrat Comment Sample Grou	Name tion Status	Succes	e nent 1 MS_25072012.m	Sample Na Position User Nam Acquired DA Metho	Vial 27 e Time 11/19/		MeO	
Compound	Table							
Common	and I alwal	RT	Mass	Farmeria			MFG Diff	
	Ind Label C16 H17 N5 O4		343.12714	Formula C16 H17 N5 O	4	MFG Formula C16 H17 N5 O4	(ppm) 2.67	DB Formula C16 H17 N5 O4
Compound	Label	m/z	RT	Algorithm	M	ass		
Cpd 7: C16 H	117 N5 O4	344.13441	0.171	Find by Molecu	lar Feature 34	43.12714		
1.5 1.25 1 0.75 0.5 0.25 0	150 200 2		C16 H18 N5 04	500 550 60	0 650 700	750 800 850	900 950	
1.25 1 0.75 0.5 0.25 0	150 200 2 m Peak List	50 300 3	350 400 450	500 550 60 vs. Mass-to-Ch		750 800 850	900 950	
1.25 1 0.75 0.5 0.25 0		50 300 3	0 350 400 450 Counts			750 800 850	900 950	
1.25 1 0.75 0.5 0.25 0 MS Spectru	m Peak List z Abund	50 300 3	0 350 400 450 Counts	vs. Mass-to-Ch		750 800 850	900 950	
1.25 1 0.75 0.5 0.25 0 MS Spectru m/z	m Peak List z Abund 1 2008	50 300 3	0 350 400 450 Counts 11a .8 N5 04	s vs. Mass-to-Ch		750 800 850	900 950	
1.25 1 0.75 0.5 0.25 0 MS Spectru <i>m/z</i> 344.13441	z Abund 1 2008 1 428	50 300 3 Formu 875.9 C16 H1	C 350 400 450 Counts 11a 18 N5 04 18 N5 04	vs. Mass-to-Ch Ion (M+H)+		750 800 850	900 950	
1.25 1 0.75 0.5 0.5 0 MS Spectru <i>m/z</i> 344.13441 345.13729	m Peak List z Abund 1 2008 1 428 1 64	50 300 3 Formu 375.9 C16 H1 315.5 C16 H1	C 350 400 450 Counts 11a 8 N5 04 8 N5 04 8 N5 04	vs. Mass-to-Ch (M+H)+ (M+H)+		750 800 850	900 950	
1.25 1 0.75 0.5 0 MS Spectru <i>m/z</i> 344.13441 345.13729 346.13909	z Abund 1 2008 1 428 1 64 1 10	Formu 75.9 C16 H1 815.5 C16 H1 921.4 C16 H1	C 400 450 Counts Ila 8 N5 04 8 N5 04 8 N5 04 8 N5 04	Ion (M+H)+ (M+H)+ (M+H)+		750 800 850	900 950	
1.25 1 0.75 0.25 0 MS Spectru <i>m/z</i> 344.13441 345.13729 346.13909 347.14035 366.1184	z Abund 1 2008 1 428 1 64 1 10	Formu 75.9 C16 H1 815.5 C16 H1 121.4 C16 H1 101.6 C16 H1 25.3 C16 H1	C 400 450 Counts Ila 8 N5 04 8 N5 04 8 N5 04 8 N5 04	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+		750 800 850	900 950	
1.25 1 0.75 0.25 0 MS Spectru <i>m/z</i> 344.13441 345.13729 346.13909 347.14035 366.1184	z Abund 1 2008 1 428 1 64 1 10 1 12	Formu 75.9 C16 H1 815.5 C16 H1 121.4 C16 H1 101.6 C16 H1 25.3 C16 H1	C 400 450 Counts Ila 8 N5 04 8 N5 04 8 N5 04 8 N5 04	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+		750 800 850		und Sum %
1.25 1 0.75 0.5 0.25 0 MS Spectru <i>m/z</i> 344.13441 345.13729 347.14035 366.1184 Predicted Is	z Abund 1 2008 1 428 1 64 1 10 1 12 sotope Matc 12	Formu 875.9 C16 H1 815.5 C16 H1 121.4 C16 H1 101.6 C16 H1 253.3 C16 H1 h Table	0 50 400 450 Counts 11a 8 N5 04 8 N5 04 8 N5 04 8 N5 04 8 N5 04 7 N5 Na 04 Diff (ppm)	Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund % C	aarge (m/z)	Abund Sum %		und Sum % 81.71
1.25 1 0.75 0.5 0.25 0 MS Spectru <i>m/z</i> 344.13441 345.13729 346.13909 347.14035 366.1184 Predicted Is Isotope	z Abund 1 2008 1 428 1 64 1 10 1 12 sotope Matc m/z	Formu 75.9 C16 H1 815.5 C16 H1 121.4 C16 H1 125.3 C16 H1 125.3 C16 H1 125.3 C16 H1 125.3 C16 H1 25.3	Counts 11a 18 N5 04 18 N5 04 18 N5 04 18 N5 04 18 N5 04 18 N5 04 18 N5 04 17 N5 Na 04 19 Diff (ppm) 33 2.66	Ion (M+H)+ (M+O)+ 6 100	aarge (m/z)	Abund Sum %	Calc Ab	
1.25 1 0.75 0.5 0.25 0 MS Spectru <i>m/z</i> 344.13441 345.13729 346.13909 347.14035 366.1184 Predicted Is Isotope 1	Abund 1 2008 1 428 1 64 1 102 sotope Matcom m/z 344.13441 345.13729	Formu 75.9 C16 H1 815.5 C16 H1 121.4 C16 H1 121.4 C16 H1 125.3 C16 H1 125.3 C16 H1 125.3 C16 H1 25.3	Image: Constraint of the second sec	Ion (M+H)+ (M+O) Abund % C 5 100 3 21.31	calc Abund %	Abund Sum %	Calc Ab 79.99	81.71

12. Compound 31

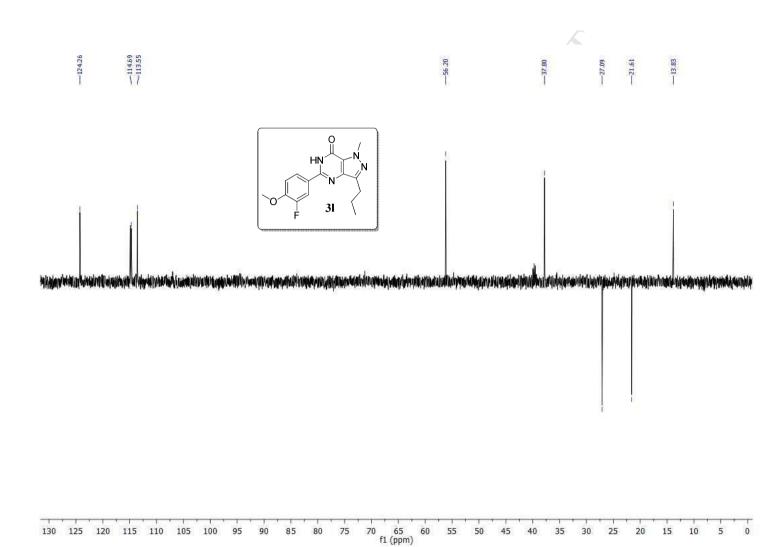


¹³C NMR in DMSO-d₆



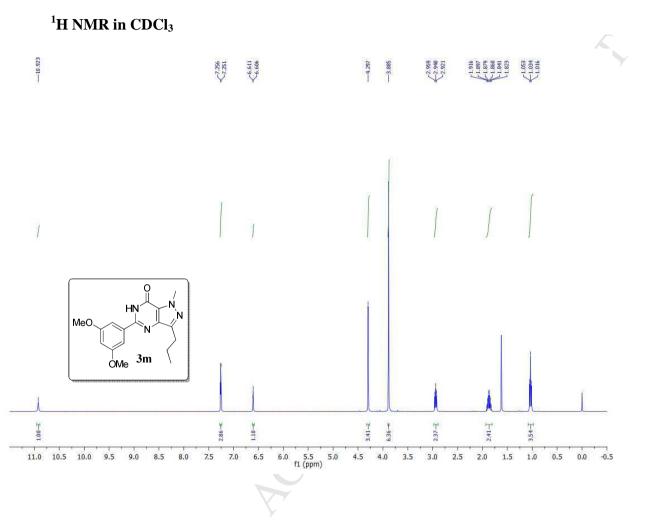
ACCEPTED MANUSCRIPT



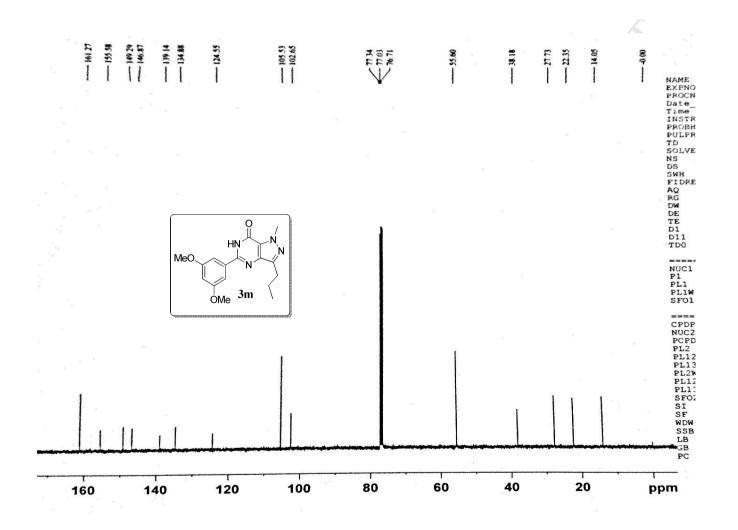


Data File Sample Type Instrument I Acq Method IRM Calibrat Comment Sample Grou	Nam tion s	Status Info	vishal Succe	le ment 1 _MS_250)72012.m	Posit User Acqui	ole Name ion Name ired Time ethod	GLR-12 Vial 21 11/19/2 as.m	012 1:58:15 PM			
											AFG Diff	
Compou		7 F N4 O2	RT 0.171	Mass 316.13		Form		-	MFG Formula	_	(ppm)	DB Formula
Cp0 5. C1	Uni	71 114 02	0.171	510.13	5296	C16 H17 F	N4 U2	C.	16 H17 F N4 O2		1.83	C16 H17 F N4 O2
Compound	Labe	el	m/z		RT	Algorithm	1	M	iss			
Cpd 5: C16 H			317.14025		0.171		lecular Fea					
6	15:0	C16 H17		MFE S	Spectrur	m (0.122-0.6	613 min) G	LR-12.d				
6 - 5 - 4 - 3 - 2 - 1 - 0 -	150	200 25	317.14025 C16 H18 F N4 O2	50 40	0 450		600 650	0 700	750 800 850	900	950	
6 5 4 3 2 1 0 	150 n Pe	200 2: eak List	000 317,14025 C16 H18 F N4 02	350 40	0 450	500 550 vs. Mass-to	600 650	0 700		900	950	
6 5 4 3 2 1 0 	150 m Pe	200 28 Pak List Abund	317,14025 317,14025 C16H18F N4 02	350 40 (0 450 Counts	500 550 vs. Mass-to	600 650 -Charge (n	0 700		900	950	
6 5 4 3 2 1 0 MS Spectrur <i>m/z</i> 317.14025	150 n Pe z	200 23 eak List Abund 71993	317.14025 317.14025 C16 H18 F N4 02 C16 H18 F N4 02	850 40 1a 8 F N4 C	0 450 Counts	500 550 vs. Mass-to Ion (M+H)-	600 650 -Charge (n	0 700		900	950	
6 5 4 3 2 1 0 	150 m Pe <u>z</u> 1	200 23 eak List Abund 71993 12777	317.14025 317.14025 24.8 C16 H1 50 02 C16 H18 F N4 02 C16 F N4 02 C17 F N4 02 C16 F N4 02 C16 F N4 02 C17 F N4 02 C16 F N	350 40 1a 8 F N4 C 8 F N4 C	0 450 Counts	500 550 vs. Mass-to Ion (M+H)- (M+H)-	600 650 -Charge (n	0 700		900	950	
6 5 4 3 2 1 0 MS Spectrur <i>m/z</i> 317.14025 318.14312	150 n Pe 1 1 1	200 23 eak List Abund 71993 12777 16	317.14025 317.14025 2008 02 216 H18 F N4 02 C16 H18 F N4 02	350 40 1a 8 F N4 C 8 F N4 C 8 F N4 C	0 450 Counts	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)-	600 650 -Charge (n	0 700		900	950	
6 5 4 3 2 1 0 MS Spectrur <i>m/z</i> 317.14025 318.14312 319.14513	150 m Pe z 1 1 1 1 1	200 25 Pak List Abund 71995 12777 16 1207	20091 2010 500 300 3 Formut 24.8 C16 H1 25.5 C16 H1 25.5 C16 H1 5543 C16 H1	la 8 F N4 C 8 F N4 C	0 450 Counts	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)+	600 650 -Charge (n	0 700		900	950	
6 5 4 3 2 1 0 MS Spectrum <i>m/z</i> 317.14025 318.14312 319.14513 320.14798 339.12253 340.12246	150 n Pee 1 1 1 1 1 1 1	200 23 ak List Abund 71993 12773 16 127 64	50 300 311214052 50 300 31121405 50 300 31121405 50 300 300 31121405 50 300 311214055 50 300 31121405 50 300 31121405 50 300 31121405 50 300 3100 50 300 300 50 300 300 50 300 300 50 300 300 50 300 300 50 30	la 8 F N4 Q 8 F N4 Q 7 F N4 N	0 450 Counts	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)-	600 650 -Charge (n	0 700		900	950	
6 5 4 3 2 1 0 MS Spectrum m/z 317.14025 318.14312 319.14513 320.14798 339.12253	150 n Pee 1 1 1 1 1 1 1	200 23 ak List Abund 71993 12773 16 127 64	50 300 311214052 50 300 31121405 50 300 31121405 50 300 300 31121405 50 300 311214055 50 300 31121405 50 300 31121405 50 300 31121405 50 300 3100 50 300 300 50 300 300 50 300 300 50 300 300 50 300 300 50 30	la 8 F N4 Q 8 F N4 Q 7 F N4 N	0 450 Counts	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)- (M+H)- (M+H)-	600 650 -Charge (n	0 700		900	950	
MS Spectrum m/z 317.14025 318.14312 319.14513 320.14798 339.12253 340.12246 Predicted Is sotope	150 n Pe z 1 1 1 1 1 1 1 1 1 1 1 1 1	200 29 ak List Abund 71993 12773 16 127 6 20 377 6 20 20 20 20 20 20 20 20 20 20	50 300 300 50 300 50 300 50 300 50 300 50 300 50 300 50 300 50 50 300 50 50 300 50 50 50 50 50 50 50 50 50 50 50 50 5	1a 8 F N4 0 8 F N4 0 8 F N4 0 8 F N4 0 8 F N4 0 7 F N4 N 7 F N4 N Diff (0 450 Counts 22 22 22 a O2 a O2	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)- (M+H)- (M+H)-	600 650 -Charge (n) 700 n/z)		900		m %
6 5 4 3 2 1 0 MS Spectrur <i>m/z</i> 317.14025 318.14312 319.14513 320.14798 339.12253 3340.12246 Predicted Is <i>isotope</i> 1	150 n Pe 1 1 1 1 1 1 1 1 1 1 1 1 1 31	200 25 ak List Abund 71993 12773 16 122 373 64 96 Match (7.14025	50 300 3 Formu 24.8 C16 H1 25.5 C16 H1 25.5 C16 H1 25.5 C16 H1 25.5 C16 H1 25.5 C16 H1 26.7 C16 H1 41.9 C16 H1 1.9 C16 H1 1.9 C16 H1 317.1408 317.1408	1a 8 F N4 Q 8 F N4 Q 8 F N4 Q 8 F N4 Q 8 F N4 Q 7 F N4 N 7 F N4 N 7 F N4 N 0 Diff (13	0 450 Counts 22 22 22 a O2 a O2	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)- (M+H)- (M+Na) (M+Na)	600 650 -Charge (n + + + + + + + + + + + + -) 700 n/z)	750 800 850 Abund Sum %	900	950 Calc Abund Su	m % 82.4
6 5 4 3 2 1 0 MS Spectrur <i>m/z</i> 317.14025 318.14312 319.14513 320.14798 339.12253 340.12246 Predicted IS <i>sotope</i> 1 2	150 n Pe <u>z</u> 1 1 1 1 1 1 1 1 1 1 1 1 1 1 31 31	200 25 200 25 200 25 201 201 201 201 201 201 201 201	200 FN J 81H 91 O 50 300 3 Formut 24.8 C16 H1 25.5 C16 H1 5543 C16 H1 5543 C16 H1 5543 C16 H1 1.9 C16 H1	1850 400 187 194 194 194 194 194 194 194 194 194 194	0 450 Counts 22 22 23 24 22 23 24 20 22 24 20 22 24 20 20 20 20 20 20 20 20 20 20 20 20 20	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)- (M+Na) (M+Na) (M+Na)	600 650 -Charge (n) 700 n/z)	750 800 850 Abund Sum %			
6 5 4 3 2 1 0 MS Spectrur <i>m/z</i> 317.14025 318.14312 319.14513 320.14798 339.12253 3340.12246 Predicted Is <i>isotope</i> 1	150 n Pe z 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	200 25 ak List Abund 71993 12773 16 122 373 64 96 Match (7.14025	50 300 3 Formu 24.8 C16 H1 25.5 C16 H1 25.5 C16 H1 25.5 C16 H1 25.5 C16 H1 25.5 C16 H1 26.7 C16 H1 41.9 C16 H1 1.9 C16 H1 1.9 C16 H1 317.1408 317.1408	13 13 14 15 15 15 15 15 15 15 15 15 15	0 450 Counts 22 22 22 22 22 22 22 22 22 22 22 22 22	500 550 vs. Mass-to (M+H)- (M+H)- (M+H)- (M+Na) (M+Na) (M+Na) (M+Na) (M+Na)	600 650 -Charge (n + + + + 0 Calc Abu) 700 n/z) 100	750 800 850	83.19		82.4

13. Compound 3m

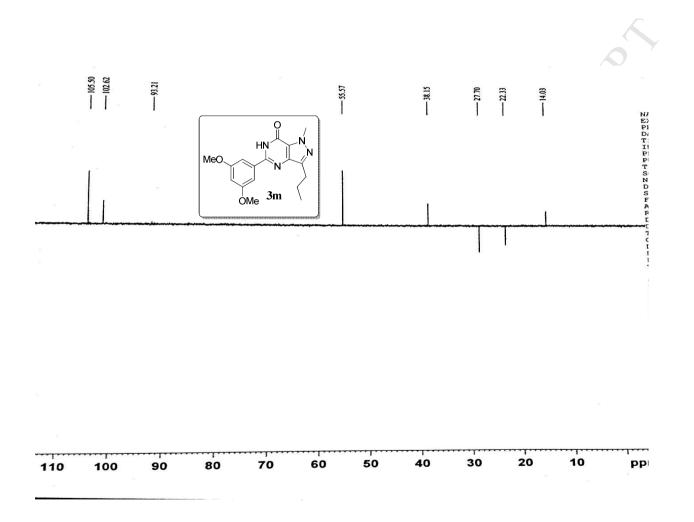


¹³C NMR in CDCl₃



ACCEPTED MANUSCRIPT

DEPT NMR in CDCl₃



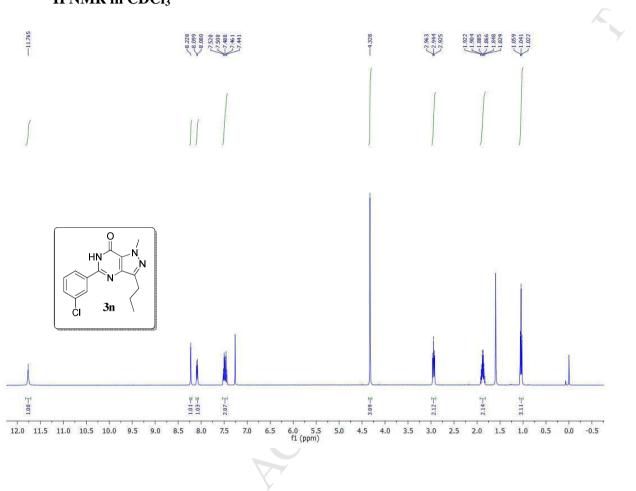
Qualitative Compound Report

Sample Typ Instrument Acq Method IRM Calibra Comment Sample Grow	Nam tion s	Status Info	vishal Succe	le ument 1 _MS_25072	2012.m	Sample N Position User Nam Acquired DA Metho	Vial ne Time 11/1	17 9/2012 1:40:29 PM		MeO	HN N Me 3m	
compound	Tab	ic			-				M	FG Diff		
Compo			RT	Mass	_	Formula		MFG Formula		(ppm)		Formula
Cpd 4:	C17 H	120 N4 O3	0.171	328.153	03	C17 H20 N4 C	03	C17 H20 N4 O3	_	1.55	C17 H	120 N4 O3
Compound	Lab	el	m/z	R	T	Algorithm		Mass	1			
Cpd 4: C17			329.1603		.171	Find by Molece	ular Feature	the second s				
x10 5 Cp 3.5 3-	id 4: (C17 H20			ctrum (0.130-0.458 m	nin) GLR-13.0	ł				
3.5- 3- 2.5- 2- 1.5- 1- 0.5-	id 4: (C17 H20	329.16031 + 1		ctrum (0.130-0.458 m	nin) GLR-13.	3				
3.5 3 2.5 2 1.5 1 0.5 0	150	200 2		05 351.14516 C17 H21 N4 03	450		00 650 70	9 0 750 800 850	0 900	950		
3.5 3 2.5 2 1.5 1 0.5 0 MS Spectru	150 um P	200 2	329.16031	00- 00- 00- 00-	450	500 550 60	00 650 70		0 900	950		
3.5- 3- 2.5- 2- 1.5- 1- 0.5-	150 Im P z	200 2: eak List	50 300	00- 00- 00- 00-	450	500 550 60 vs. Mass-to-Cr	00 650 70		0 900	950		
3.5 3.2 2.5 2.5 1.5 1 0.5 0 MS Spectru <i>m/z</i> 329.16031 330.1629	150 Im P z 1 1 9 1	200 2 eak List Abund 3873	50 300	81 351.14516 C17 H21 N4 03 00 351.14516 C17 H21 N4 03 000 000 000	450	500 550 60 vs. Mass-to-CP Ion	00 650 70		0.00	950		
3.5 3 2.5 2 1.5 1 0.5 0 MS Spectru <i>m/z</i> 329.16031 330.1625 331.16576	150 JIM P Z 1 1 1 5 1	200 2 eak List 3873 813 84	50 300 Form 20.4 C17 H 72.2 C17 H 49.1 C17 H	align 351.14516 C17 H21 N4 03 700 P 00 351.14516 017 H21 N4 03 700 P 00 00 00 700 P 00 00 00	450	500 550 60 vs. Mass-to-Cf (M+H)+ (M+H)+ (M+H)+	00 650 70		0.00	950		
3.5 3 2.5 2 1.5 1 0.5 0 MS Spectru <i>m/z</i> 329.16031 330.1625 331.16576 332.16928	150 Jum P z 1 1 1 5 1 3 1	200 2 eak List Abund 3873 813 84 14	50 300 Form 20.4 C17 F 77.2 C17 F 49.1 C17 F 78.8 C17 F	align 351.14516 C17 H21 N4 03 700 P 00 351.14516 017 H21 N4 03 700 P 00 00 00 700 P 00 00 00	450	500 550 66 vs. Mass-to-Cf (M+H)+ (M+H)+ (M+H)+ (M+H)+	00 650 70		0 900	950		
3.5 3.2 2.5 2.5 1.5 1.5 0.5 0.5 0.5 329.16031 330.1625 331.16576 332.16928 351.14516	150 IT P Z 1 1 1 3 1 5 1 5 1	200 2 eak List Abund 3873 813 844 14 27	50 300 Form 20.4 C17 H 72.2 C17 H 78.8 C17 H 78.8 C17 H 54.7	align 351.14516 C17 H21 N4 03 700 P 00 351.14516 017 H21 N4 03 700 P 00 00 00 700 P 00 00 00	450	500 550 60 vs. Mass-to-Cf (M+H)+ (M+H)+ (M+H)+	00 650 70		0 900	950		
3.5 3.2.5 2.5 1.5 1.5 0.5 0.5 0.5 329.16031 330.1625 331.16576 332.16926 332.16926 335.114516	150 IM P Z 1 3 1 5 1 Soto	200 2 eak List Abund 3873 813 84 14 27 pe Matci	50 300 Form 20.4 C17 F 72.2 C17 F 49.1 C17 F 78.8 C17 F 54.7 h Table	C 211 H421 03 C 211 H421 04 C 212	450 counts	500 550 66 vs. Mass-to-Cf (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+	00 650 70 harge (m/z)	0 750 800 850				
3.5 3.2 2.5 1.5 1.5 0.5 329.16031 330.1629 331.16576 332.16928 351.14516 Predicted I Isotope	150 Im P Z 1 1 1 1 1 1 Soto m/z	200 2 eak List Abund 3873 813 84 14 27 pe Matcl	50 300 Form 20.4 C17 H 72.2 C17 H 49.1 C17 H 78.8 C17 H 54.7 h Table Calc m/z	UIA 14219 UIA 14219	450 counts	500 550 66 vs. Mass-to-Cr (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund %	00 650 70	0 750 800 850	/0	950	Sum %	
3.5 3.2 2.5 2.5 1.5 1.5 0.5 329.16031 330.1629 331.16576 332.16926 332.145016 Predicted I Isotope	1500 Im P Z 1 1 1 1 1 1 Soto m/2 3	200 2 eak List Abund 3873 813 84 14 27 pe Matcl 29.16031	50 300 Form 20.4 C17 H 72.2 C17 H 49.1 C17 H 78.8 C17 H 54.7 h Table Calc m/z 329.160	C 11 H4 03 21 H4 03 2	2 450 counts 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	500 550 60 vs. Mass-to-Cr (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund % (100)	00 650 70 arge (m/z)	0 750 800 850	% 80.92	Calc Abund		81.3
3.5 3.2 2.5 1.5 1.0 5 329.16031 330.1629 331.16576 332.16926 351.14516 Predicted I Isotope	1500 Im P Z 1 1 1 1 1 Soto m/2 2	200 2 eak List Abund 3873 813 84 14 27 pe Matcl	50 300 Form 20.4 C17 H 72.2 C17 H 49.1 C17 H 78.8 C17 H 54.7 h Table Calc m/z	UIA 103 221'14218 221'14218 221'14218 221'14203 221 N4 03 221 N4 03 231 N5	450 counts	500 550 66 vs. Mass-to-Cr (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund %	00 650 70 harge (m/z)	0 750 800 850	/0	Calc Abund		81.3 16.43 2.08

---- End Of Report ----

14. Compound 3n

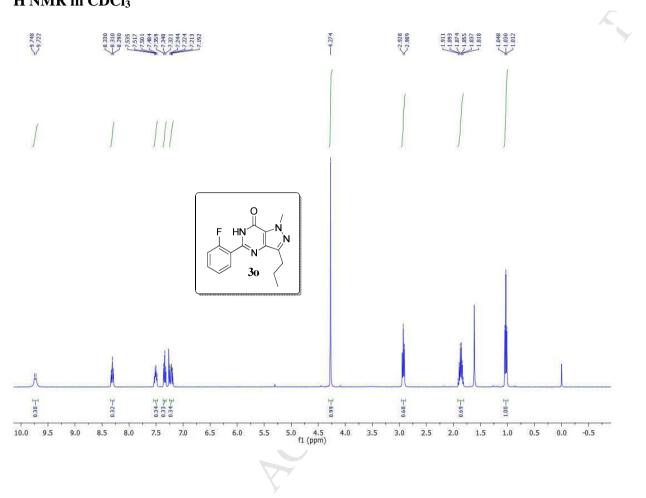




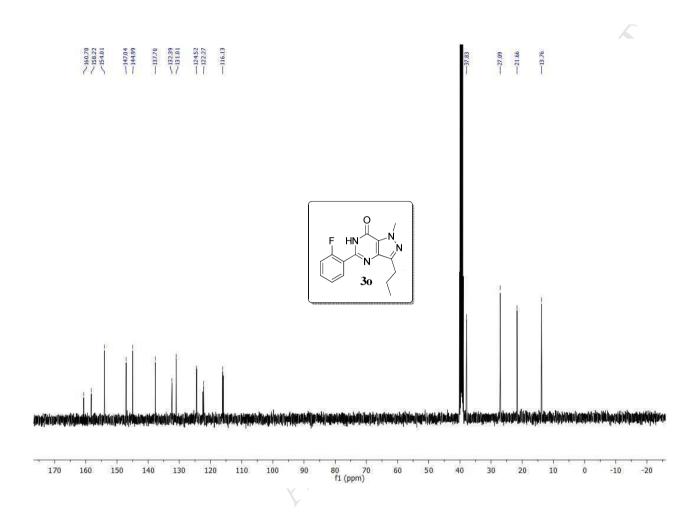
Sample Type Instrument Acq Method IRM Calibra Comment Sample Grou	Name tion Status	Success	ent 1 15_25072012.m	Position User Na	ame ed Time 11/19		CI	HN N N 3n
Compound	1.					-	MFG Diff	
	und Label	RT 0.172	Mass 302.09267	Formula C15 H15 CI		MFG Formula C15 H15 CI N4 O	(ppm) 2.55	DB Formula C15 H15 CI N4 C
Compound Cpd 4: C15 H		<i>m/z</i> 303.09995	RT 0.172	Algorithm Find by Mole	ecular Feature	Aass 02.09267		
x10 ⁵ Cp 3- 2.5- 2-	d 4: C15 H15		MFE Spectrun	n (0.131-0.47	5 min) GLR-14.	d		
3- 2.5- 2- 1.5- 1- 0.5-	d 4: C15 H15	303.09995 C15 H16 CI N4 O C15 H16 CI N4 O	I/FE Spectrun	n (0.131-0.47	5 min) GLR-14.	d		
3- 2.5- 2- 1.5- 1- 0.5- 0-	150 200 2	303.09995 50 005 015 H16 Cl N4 O	50 400 450	n (0.131-0.47) 500 550 vs. Mass-to-(600 650 70		00 950	
3- 2.5- 2- 1.5- 1- 0.5- 0-		303.09995 50 005 015 H16 Cl N4 O	50 400 450 Counts	500 550	600 650 70		00 950	
3- 2.5- 2- 1.5- 1- 0.5- 0 MS Spectru	150 200 2 m Peak List z Abund	303.09995 52 005 055 515 H16 Cl N4 O	50 400 450 Counts	500 550 vs. Mass-to-0	600 650 70		00 950	
3- 2.5- 2- 1.5- 1- 0.5- 0- MS Spectru <i>m/z</i> 303.09995 304.10235	150 200 2 m Peak List 2 Abund 1 3277 1 567	0 HN 0 96660 200 39 250 300 39 Formula 25.1 C15 H16 78.1 C15 H16 78.1 C15 H16	50 400 450 Counts a CI N4 0 CI N4 0	500 550 vs. Mass-to-(Ion	600 650 70		00 950	
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 305.09724	150 200 2 m Peak List 2 Abund 1 3277 1 567 1 1082	250 300 33 Formula 25.1 C15 H16 78.1 C15 H16 61.9 C15 H16	50 400 450 Counts Cl N4 0 Cl N4 0 Cl N4 0	500 550 vs. Mass-to-0 Ion (M+H)+	600 650 70		00 950	
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 305.09724 306.09989	150 200 2 m Peak List 2 Abund 1 3277 1 567 1 1082 1 192	250 300 33 Formula 251 C15 H16 78.1 C15 H16 61.9 C15 H16 60.9.3 C15 H16	50 400 450 Counts Counts Cl N4 0 Cl N4 0 Cl N4 0 Cl N4 0	500 550 vs. Mass-to-(Ion (M+H)+ (M+H)+	600 650 70		00 950	
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 305.09724 306.09989 307.10223	150 200 2 m Peak List 1 3277 1 567 1 1082 1 192 1 17	250 300 33 Formula 25.1 C15 H16 78.1 C15 H16 61.9 C15 H16	50 400 450 Counts Counts Cl N4 0 Cl N4 0 Cl N4 0 Cl N4 0	500 550 vs. Mass-to-([[(M+H)+ (M+H)+ (M+H)+	600 650 70		00 950	
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 305.09724 306.09989 307.10223 325.08122	150 200 2 m Peak List 1 3277 1 567 1 1082 1 192 1 177 1 20	250 300 3 Formula 25.1 C15 H16 178.1 C15 H16 109.3 C15 H16 109.3 C15 H16 184.4 C15 H16 184.4 C15 H16	50 400 450 Counts CI N4 0 CI N4 0 CI N4 0 CI N4 0 CI N4 0 CI N4 0	500 550 vs. Mass-to-((M+H)+ (M+H)+ (M+H)+	600 650 700 Charge (m/z)		00 950	
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 304.10235 305.09724 306.09889 307.10223 307.10223 325.08122 Predicted I	150 200 2 m Peak List 1 3277 1 567 1 1082 1 192 1 177 1 20 sotope Matc	Formula 250 300 39 250 300 39 251 C15 H16 39 252.1 C15 H16 30 253.1 C15 H16 30 309.3 C15 H16 30 309.3 C15 H16 30 302.6 C15 H15 10 302.6 C15 H15 10	50 400 450 Counts CI N4 0 CI N4 0 CI N4 0 CI N4 0 CI N4 0 CI N4 0	500 550 vs. Mass-to-((M+H)+ (M+H)+ (M+H)+ (M+H)+	600 650 700 Charge (m/z)		00 950	
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 304.10235 305.09724 306.09989 307.10223 325.08122 Predicted II Isotope	150 200 2 m Peak List 2 Abund 1 3277 1 567 1 1082 1 192 1 17 1 20 sotope Matci m/z	250 300 33 Formula 25.1 C15 H16 72.1 C15 H16 66.1.9 C15 H16 78.4 C1	50 400 450 Counts a Cl N4 0 Cl	500 550 vs. Mass-to-([M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund %	600 650 700 Charge (m/z)		00 950	Sum %
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 305.09724 306.09889 307.10223 307.1023 3	150 200 2 m Peak List 2 Abund 1 3277 1 567 1 1082 1 192 1 177 1 200 sotope Matco sotope Matco m/z 303.09995	Formula 250 300 39 250 300 39 251 C15 H16 39 252.1 C15 H16 30 253.1 C15 H16 30 309.3 C15 H16 30 309.3 C15 H16 30 302.6 C15 H15 10 302.6 C15 H15 10	50 400 450 Counts a Cl N4 0 Cl N4 0 Diff (ppm)	500 550 vs. Mass-to-((M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+	600 650 700 Charge (m/z)	0 750 800 850 9		Sum % 63.28
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 305.09724 306.09989 307.10223 305.09724 Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution	150 200 2 m Peak List 2 Abund 1 3277 1 567 1 1082 1 1082 1 192 1 177 1 20 sotope Matco m/z 303.09995 304.10235	250 300 33 Formula 25.1 C15 H16 72.1 C15 H16 66.1.9 C15 H16 78.4 C1	50 400 450 Counts a Cl N4 0 Cl	500 550 vs. Mass-to-([M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund %	600 650 700 Charge (m/z)	0 750 800 850 s	Calc Abund	
3- 2.5- 2- 1.5- 1- 0.5- 0- MS Spectrum m/z 303.09995 304.10235 305.09724 306.09989 307.10223 325.08122 Predicted II Isotope 1 2 3 3 3 2 3 3 2 3 3 2 3 3 3 3 3 3 3 3 3 3 3 3 3	150 200 2 m Peak List z Abund 1 3277 1 567 1 1082 1 1082 1 1082 1 1082 1 1082 1 1082 3 1082 1 30309995 304.10235 305.09724	250 300 33 Formula 25.1 C15 H16 78.1 C15 H16 66.1.9 C15 H16 78.4 C1	50 400 450 Counts Cl N4 0 Cl N4 Na 0 Diff (ppm) 2.51 4.08	500 550 vs. Mass-to-((M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund % 100	600 650 700 Charge (m/z)	Abund Sum % 00 63 91 11	Calc Abund	63.28
3 - 2.5 - 2 - 1.5 - 1 - 0.5 - 0 - MS Spectru <i>m/z</i> 303.09995 304.10235 305.09724 306.09989 307.10223 305.09724 Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution Solution	150 200 2 m Peak List z Abund 1 3277 1 567 1 1082 1 192 1 192 1 192 1 171 1 200 sotope Matci m/z 303.09995 304.10235 305.09724 306.09989 304.09989	250 300 33 Formula 251 C15 H16 78.1 C15 H16 78.1 C15 H16 78.1 C15 H16 79.1 C15 H	50 400 450 Counts Counts CI N4 0 CI SI	500 550 vs. Mass-to-((M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)+ Abund % 100 17.32	600 650 700 Charge (m/z)	Abund Sum % 0 750 800 850 9 0 63 91 11 71 21	Calc Abund	63.28 11.33

15. Compound 3o

¹H NMR in CDCl₃

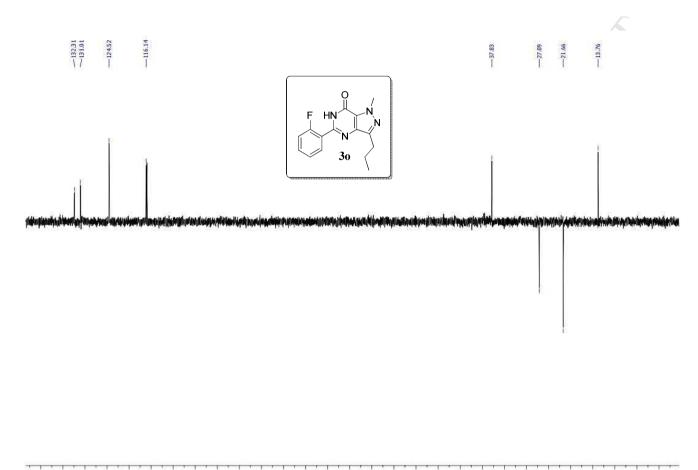






ACCEPTED MANUSCRIPT

DEPT NMR in DMSO-d₆



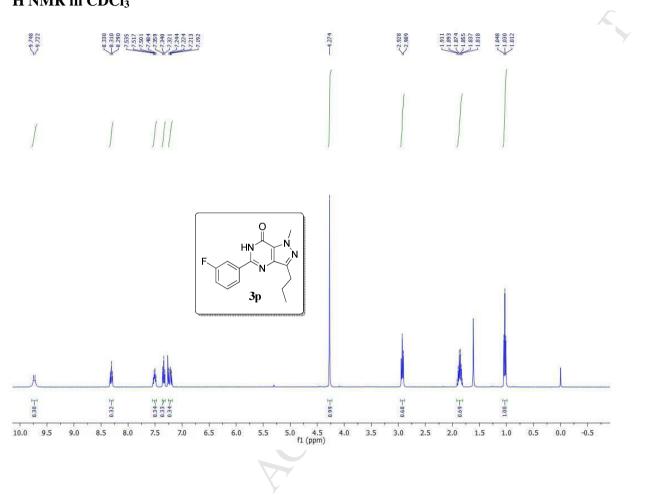
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 f1 (ppm)

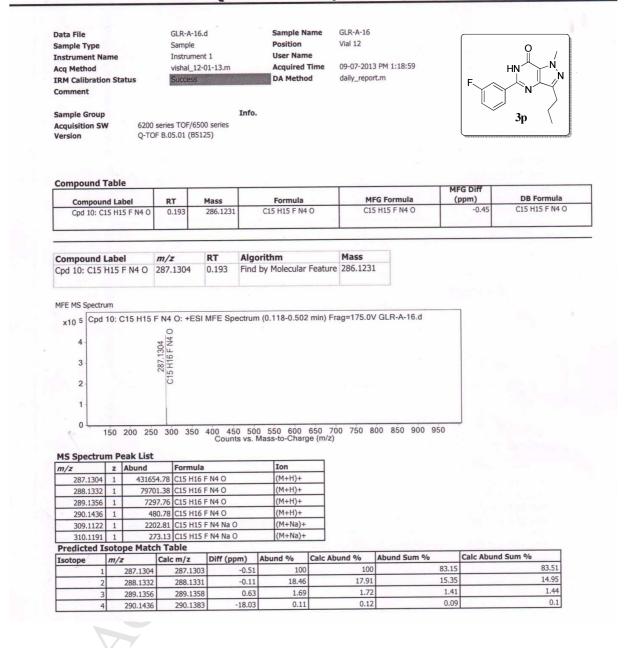
Qualitative Compound Report

Sample Type Instrument N Acq Method IRM Calibrati Comment Sample Grou	on Status	In: vis	imple strument 1 shal_MS_2 iccess	5072012.m	Position User Nam Acquired DA Metho	Time 11/1	9/2012 2:15:56 PM			
Compound	Table						فالمراجع والم	M	GDiff	
Compound Label		RT	RT Mas		ss Formula		MFG Formula		opm)	DB Formula
Cpd 64: C15 H15 F N4 C				.12217	C15 H15 F N4	0	C15 H15 F N4 O		2.87	C15 H15 F N4
	lahal			RT	Algorithm		Mass	-		
Compound Label Cpd 64: C15 H15 F N4 O		m/z	287.12944		Find by Molec	ular Feature				
1.2		287.12944 C15.116.6 MA O								
1- 0.8- 0.6- 0.4- 0.2- 0-										
0.8 0.6 0.4 0.2	150 200 m Peak Li	250 30	00 350	400 450 Counts	500 550 (vs. Mass-to-C	600 650 70 harge (m/z)	00 750 800 85	50 900	950	
0.8 0.6 0.4 0.2 0	m Peak Li z Abund	250 30 st	ormula		Ion	600 650 7(harge (m/z)	00 750 800 85	50 900	950	
0.8 0.6 0.4 0.2 0 MS Spectru	m Peak Li	250 30 st 5751.1 C	00 350 ormula 15 H16 F I	N4 O	Ion (M+H)+	300 650 7(harge (m/z)	00 750 800 85	50 900	950	
0.8 0.6 0.4 0.2 0 MS Spectru <i>m/z</i>	m Peak Li	250 30 st 5751.1 C 2258.4 C	00 350 ormula 15 H16 F I 15 H16 F I	14 0 14 0	Ion (M+H)+ (M+H)+	300 650 7(harge (m/z)	00 750 800 85	50 900	950	
0.8 0.6 0.4 0.2 0 MS Spectru <i>m/z</i> 287.12944 288.13243 289.13631	m Peak Li	250 30 st 5751.1 C 2258.4 C 3908.1 C	00 350 ormula 15 H16 F I 15 H16 F I 15 H16 F I	14 0 14 0	Ion (M+H)+	500 650 7(harge (m/z)	0 750 800 85	50 900	950	
0.8 0.6 0.4 0.2 0 MS Spectru <i>m/z</i> 287.12944 288.13243 289.13631 Predicted I	m Peak Li	250 30 st 55751.1 C 22258.4 C 3908.1 C tch Tab	ormula 15 H16 F I 15 H16 F I 15 H16 F I 15 H16 F I 15 H16 F I	14 0 14 0 14 0	Ion (M+H)+ (M+H)+ (M+H)+					
0.8 0.6 0.4 0.2 0 MS Spectru <i>m/z</i> 287.12944 288.13243 289.13631 Predicted I Isotope	m Peak Li z Abund 1 10 1 sotope Ma m/z	250 30 st 5751.1 C 2258.4 C 3908.1 C tch Tab Calc n	ormula 15 H16 F f 15 H16 F f 15 H16 F f 15 H16 F f 15 H16 F f le n/z D	14 0 14 0 14 0 iff (ppm)	Ion (M+H)+ (M+H)+ (M+H)+	500 650 70 harge (m/z) Calc Abund G	% Abund Sum	%	950 Calc Abune	
0.8 0.6 0.4 0.2 0 MS Spectru <i>m/z</i> 287.12944 288.13243 289.13631 Predicted I	z Abund 1 10 1 10 1 10 sotope Ma m/z 287.129 287.129	250 30 5751.1 C 12258.4 C 3908.1 C tch Table Calc m 44 283	ormula 15 H16 F I 15 H16 F I 15 H16 F I 15 H16 F I 15 H16 F I	14 0 14 0 14 0	Ion (M+H)+ (M+H)+ (M+H)+ Abund % 100	Calc Abund ⁶				d Sum % 83.59 14.97

16. Compound 3p

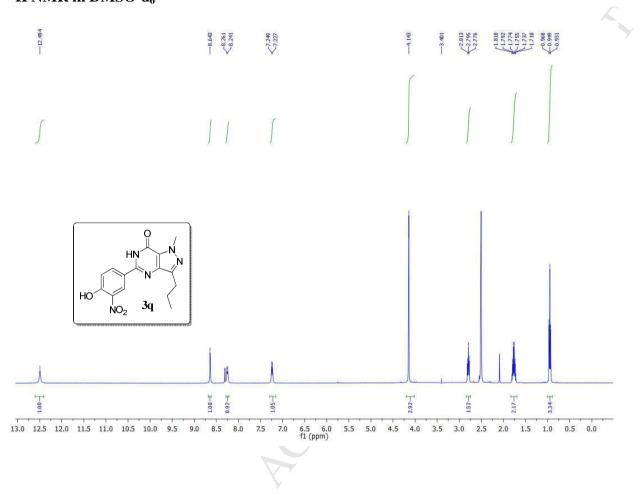
¹H NMR in CDCl₃



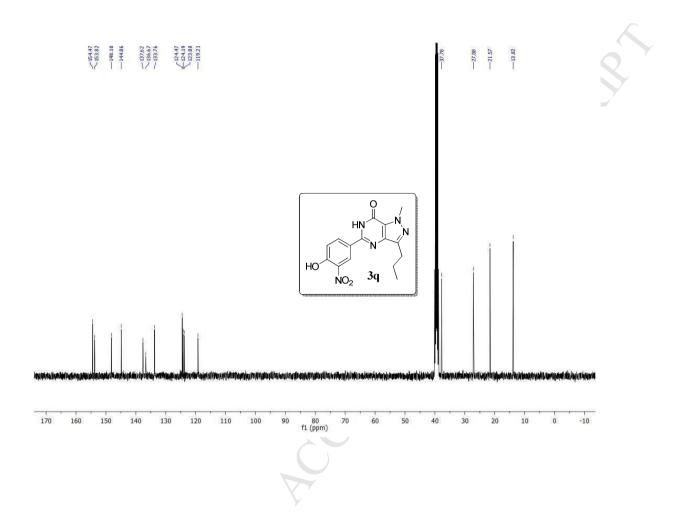


17. Compound 3q

¹H NMR in DMSO-d₆

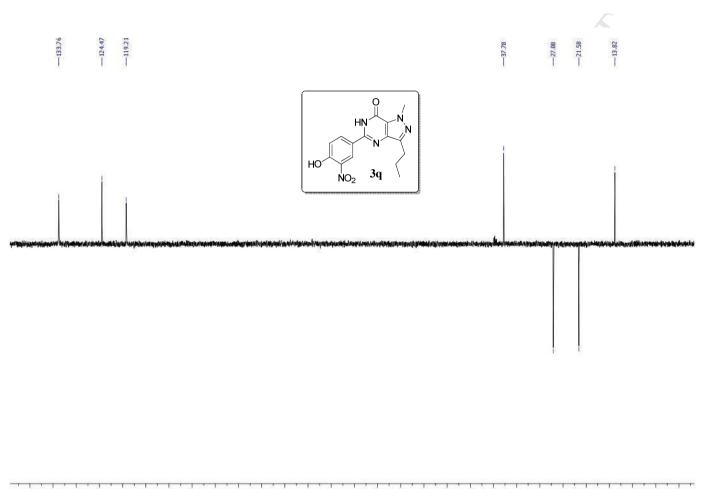






ACCEPTED MANUSCRIPT





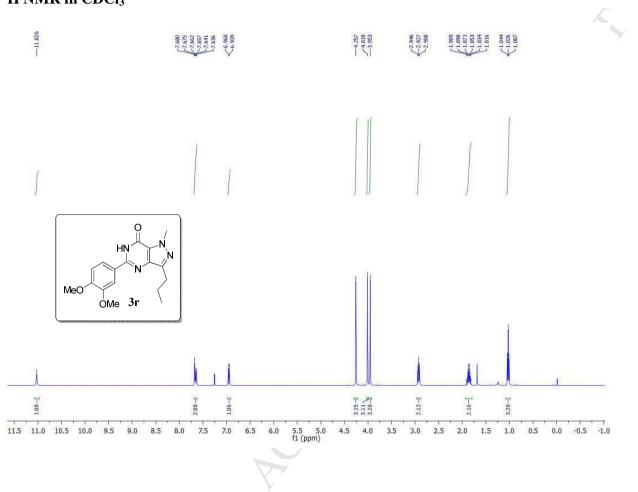
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 f1 (ppm)

Acq Method IRM Calibrati Comment		Success	s information is u	Acquired DA Metho unavailable		HONO	NO ₂ 3q	
Compound 1				-		MFG Diff	DB Farmula	
Compound Label Cpd 70: C15 H15 N5 O4		RT 0.568	Mass 329.11153	Formula C15 H15 N5 O4	C15 H15 N5 O4	(ppm) 2.67	DB Formula C15 H15 N5 O4	
Compound Label		<i>m/z</i> 330.11878	RT	Algorithm	Mass			
Cpd 70: C15	Cpd 70: C15 H15 N5 O4		0.568	Find by Molecular Featu	re 329.11153			
x10 4 Cpc	1 /0: C15 H1	5 N5 04: +	MFE Spectru	m (0.186-0.628 min) GLR	-17.0			
1.75 1.5 1.25 1 0.75 0.5 0.25			330.11878 C15 H16 N5 O4					
1.5 - 1.25 - 1 - 0.75 - 0.5 - 0.25 - 0 -			330.11878 330.11878 330.11878 330.11878 330.11878	0 375 400 425 450 475 s vs. Mass-to-Charge (m/	5 500 525 550 575 60	00 625 650		
1.5 1.25 1 0.75 0.5 0.25	m Peak List		330.11878 330.11878 330.11878 330.11878 330.11878	0 375 400 425 450 475	5 500 525 550 575 60	0 625 650		
1.5 1.25 1 0.75 0.5 0.25 0 MS Spectru	m Peak List z Abund		300 325 350 Count) 375 400 425 450 475 s vs. Mass-to-Charge (m/	5 500 525 550 575 60	00 625 650		
1.5- 1.25- 1- 0.75- 0.25- 0- MS Spectru <i>m/z</i>	m Peak List z Abund 1 19252.1	Formula	300 11878 Cont Cont 230 11878	0 375 400 425 450 475 s vs. Mass-to-Charge (m/	5 500 525 550 575 60	0 625 650		

Isotope	m/z	Calc m/z	Diff (ppm)	Abund %	Calc Abund %	Abund Sum %	Calc Abund Sum %
	330.11878			100	100	70.63	82.78
	331.12188	331.12244	1.71	27.86	18.39	19.68	15.22
	3 332.13058	332.12475	-17.55	13.72	2.42	9.69	
	8	5					

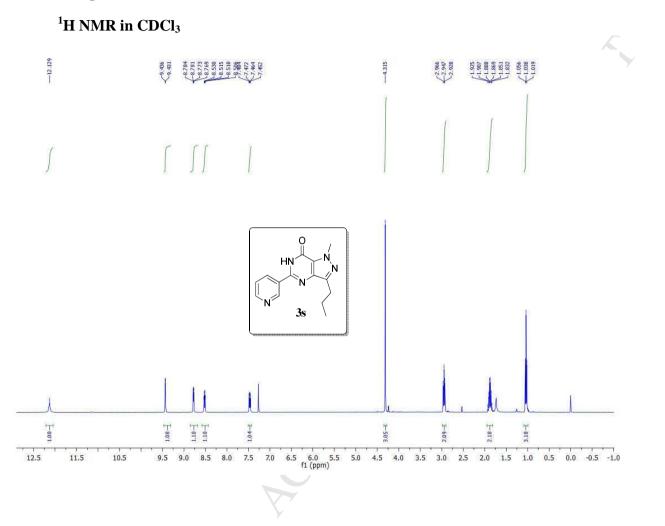
18. Compound 3r

¹H NMR in CDCl₃



(ppm) DB Formula 1.53 C17 H20 N4 03			C1 Mas	Formula C17 H20 N4 O3 Igorithm nd by Molecula	15304 RT		RT 0.171	H20 N4 O3	mpound L od 5: C17 H	Cpr Compos
1.53 C17 H20 N4 03		ISS	Mas	lgorithm	RT					Compo
			and the second se	and the second s			m/z	bel	ound Labo	
	-		and the second se	and the second s			m/z	bel	und Lab	
	-	8.15304	Feature 328.	nd by Molecula	0.171					
	- 42					32	329.1603	N4 O3	C17 H20 N	Cpd 5: C
	96									
	110								Casada	
				120 0 401 min	nootrum (MEE S	14 03. +		Spectrum	
			GLR-18.0	130-0.491 min	spectrum (10.55		C17 H201	Cpu 5. (x10 ⁵
						03	~			3.5
						N4	03			
						21	.16			
the second se						HL	329			
						G			-	2
									-	1.5
						-				
							_			
							_			0.5
900 950	0 000 0	750 800 850	650 700 7	00 550 600	400 450	350 4	50 300	0 200 25	150	0-
300 330	5 300 3	/00 000 000	e (m/z)	Mass-to-Char	Counts v					
								Peak List	ectrum Pe	MS Spe
				Ion		nula	Form	Peak List Abund		MS Spe m/z
				Ion (M+H)+	03		Form	Abund	z	
				the second se		H21 N4 0		Abund 40904	z .6032 1	m/z
				(M+H)+	03	H21 N4 O	2.8 C17 H	Abund 40904 8140	z 6032 1 6284 1	m/z 329.16
				(M+H)+ (M+H)+	03 03	H21 N4 0 H21 N4 0 H21 N4 0	12.8 C17 H	Abund 40904 8140 1114	2 6032 1 6284 1 .1662 1	m/z 329.16 330.16
				(M+H)+ (M+H)+ (M+H)+	03 03	H21 N4 0 H21 N4 0 H21 N4 0	H2.8 C17 H 08.9 C17 H H1.1 C17 H 11.2 C17 H	Abund 40904 8140 1114	z .6032 1 .6284 1 .1662 1 .6535 1	m/z 329.16 330.16 331.1 332.16
Calc Abund Sum %	6	Abund Sum %	Abund %	(M+H)+ (M+H)+ (M+H)+ (M+H)+	03 03 03	H21 N4 0 H21 N4 0 H21 N4 0 H21 N4 0 H21 N4 0	H2.8 C17 H 08.9 C17 H H1.1 C17 H 11.2 C17 H	Abund 40904 8140 1114 121 ope Match	2 6032 1 6284 1 1662 1 6535 1 ed Isoto	m/z 329.16 330.16 331.1 332.16
Calc Abund Sum %			Abund %	(M+H)+ (M+H)+ (M+H)+ (M+H)+	03 03 03	H21 N4 0 H21 N4 0 H21 N4 0 H21 N4 0 H21 N4 0	H2.8 C17 H 08.9 C17 H H1.1 C17 H 11.2 C17 H I Table	Abund 40904 8140 1114 121 ope Match	2 1 1 1 1 1 1 1 1 1 1 1 1 1	m/z 329.16 330.16 331.1 332.16 Predicte
	81.35			(M+H)+ (M+H)+ (M+H)+ (M+H)+	03 03 03 f (ppm)	H21 N4 0 H21 N4 0 H21 N4 0 H21 N4 0 H21 N4 0 H21 N4 0	12.8 C17 H 13.9 C17 H 11.1 C17 H 11.2 C17 H 11.2 C17 H 1 Table Calc m/z	Abund 40904 8140 11114 121 0pe Match 7z	2 16032 1 16284 1 1662 1 16535 1 1 1 1 1 1 3 1 3	m/z 329.16 330.16 331.1 332.16 Predicte
81.35 81.3	81.35 16.19	2	100	(M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ 2000 % Cal 100	03 03 03 f (ppm) 1.51	H21 N4 0 H21	42.8 C17 H 18.9 C17 H 11.1 C17 H 11.2 C17 H 11.2 C17 H 1 Table Calc m/z 329.160	Abund 40904 8140 11114 121 ope Match 'z 329.16032	z .6032 1 .6284 1 .1662 1 .6535 1 .eed Isoto m/z 1 3 2 3	m/z 329.16 330.16 331.1 332.16 Predicte
						C17 H21 N4 03	329.16032		-	3 2.5 1.5 1 0.5

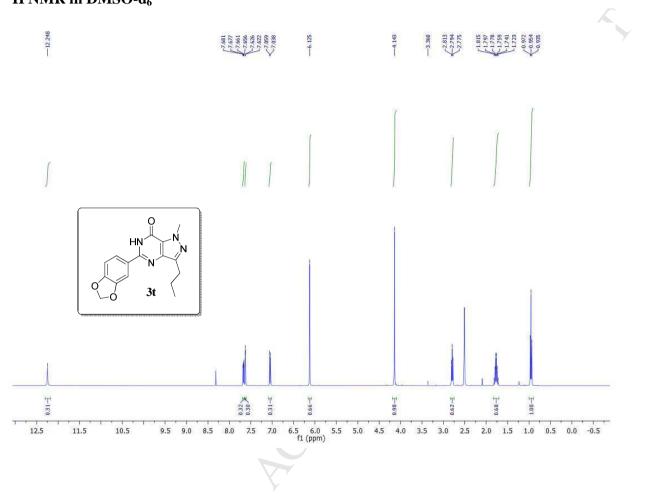
19. Compound 3s



Instrument M Acq Method IRM Calibrat Comment Sample Grou	ion :		Sa Ins DA	ily ms0: imple strumer AILY MS Tons M	nt 1 DESI.m	Sample Positio User Ni Acquire DA Met	n 6 ame ed Time 2		2 5:05:14 PM		N ⁻	$ \begin{array}{c} $	
Compound	Tab	le											
Compou	und I	ahel	RT		Mass	Formula		M	IFG Formula	MFG Diff (ppm)		DB Formula	
		H15 N5 O			69.12776	C14 H15 N			14 H15 N5 O		-0.36	C14 H15 N5 O	
										-			
Compound			m/z		RT	Algorithm		Ma	the second se				
Cpd 1: C14 H	115 1	V5 O	270.13	503	0.121	Find by Mole	ecular Featu	re 269	0.12776				
6 -		270.13503 C14 H16 N5 O											
4-	200	0 250	300 3	50 40		00 550 60 vs. Mass-to-	0 650 70 Charge (m/z	00 75 z)	0 800 850 s	900 9	50		
4 2 0 MS Spectru	m P	0 250	300 3	50 40	00 450 5 Counts	00 550 60 vs. Mass-to-	0 650 70 Charge (m/2	00 75 z)	0 800 850 s	900 9	50		
4 2 0 MS Spectru	m P	250 eak List Abund	300 3	rmula	Counts	vs. Mass-to-	Charge (m/z	00 75 z)	0 800 850 s	900 9	50		
4 2 0 MS Spectru <i>m/z</i>	m P	250 eak List Abund 9193	300 3	rmula 4 H16 M	Counts	vs. Mass-to-	Charge (m/z	00 75 z)	0 800 850 s	900 9	50		
4- 2- 0 MS Spectru <i>m/z</i> 270.13503 271.13807 272.14014	m P z 1 1	250 eak List Abund 9193 1781 139	300 3 Fo 37.8 C1 31.8 C1 54.7 C1	rmula 4 H16 M 4 H16 M 4 H16 M	Counts 15 0 15 0 15 0	vs. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+	Charge (m/z	00 75 z)	0 800 850 s	900 9	50		
4 - 2 - 0 - MS Spectru m/z 270.13503 271.13807 272.14014 273.14255	m P z 1 1 1 1	250 eak List Abund 9193 1781 139 8	300 3 Fo 37.8 C1 31.8 C1 54.7 C1 22.6 C1	ermula 4 H16 M 4 H16 M 4 H16 M 4 H16 M	Counts 15 0 15 0 15 0 15 0	vs. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+	Charge (m/z	00 75 z)	0 800 850 S	900 9	50		
4 - 2 - 0 - MS Spectru m/z 270.13503 271.13807 272.14014 273.14255 292.11731	m P z 1 1 1 1 1	250 eak List Abund 9193 1781 139 8 46	300 3 Fo 337.8 C1 31.8 C1 31.8 C1 54.7 C1 22.6 C1 81.6 C1	ermula 4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H16 M	Counts 45 0 45 0 45 0 45 0 45 0 45 0 45 0	vs. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)-	Charge (m/2	00 75 z)	0 800 850 S	900 9	50		
4 - 2 - 0 - MS Spectru <i>m/z</i> 270.13503 271.13807 272.14014 273.14255 292.11731 293.12003	m P z 1 1 1 1 1 1 1	250 eak List Abund 9193 1781 139 8 46 9	300 3 37.8 C1 37.8 C1 31.8 C1 54.7 C1 54.7 C1 54.7 C1 54.6 C1 88.6 C1	rmula 4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H15 M 4 H15 M	Counts 15 0 15 0 15 0 15 0 15 0 15 Na 0 15 Na 0	vs. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)-	Charge (m/2	00 75 z)	0 800 850 S	900 9	50		
4 2- 0 MS Spectru <i>m/z</i> 270.13503 271.13807 272.14014 272.14014 273.14255 292.11731 293.12003 308.08798	m P z 1 1 1 1 1 1 1 1 1	250 eak List Abund 9193 1781 1781 139 8 8 46 9 76	300 3 37.8 C1 31.8 C1 54.7 C1 22.6 C1 81.6 C1 88.6 C1 53.7 C1	ermula 4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H15 M 4 H15 M 4 H15 M	Counts 15 0 15 0 15 0 15 0 15 Na 0 15 Na 0 15 Na 0 15 Na 0	VS. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)- (M+K)+	Charge (m/2	00 75 z)	0 800 850 s	900 9	50		
4 2- 0 MS Spectru <i>m/z</i> 270.13503 271.13807 272.14014 273.14255 279.11731 293.12003 308.08798 309.09111	m P z 1 1 1 1 1 1 1 1 1 1	250 eak List Abund 9193 1781 135 8 46 9 9 76 16	300 3 37.8 C1 37.8 C1 31.8 C1 22.6 C1 81.6 C1 88.6 C1 53.7 C1 44.3 C1	rmula 4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M	Counts 15 0 15 0 15 0 15 0 15 Na 0 15 Na 0 15 Na 0 15 Na 0 15 No 0	VS. Mass-to- (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)- (M+K)+ (M+K)+	Charge (m/2	00 75 ⁽ z)	0 800 850 s	900 99	50		
4 2 0 MS Spectru <i>m/z</i> 270.13503 271.13807 272.14014 273.14255 292.11731 293.12003 308.08798 309.09111 310.08961	m P z 1 1 1 1 1 1 1 1 1 1 1 1 1 1	250 eak List Abund 9193 1781 139 8 46 9 766 166	300 3 37.8 C1 31.8 C1 54.7 C1 22.6 C1 88.6 C1 88.6 C1 53.7 C1 44.3 C1 44.3 C1 44.3 C1	rmula 4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M	Counts 15 0 15 0 15 0 15 0 15 Na 0 15 Na 0 15 Na 0 15 Na 0 15 No 0	VS. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)- (M+K)+	Charge (m/2	00 75 z)	0 800 850 S	900 9:	50		
4 2 0 MS Spectru m/z 270.13503 271.13807 272.14014 273.14255 292.11731 293.12003 308.08798 309.09111 310.08961 Predicted Is	m P z 1 1 1 1 1 1 1 1 1 1 1 1 5000	250 eak List Abund 9193 1781 139 8 46 9 76 16 6 6 6 6 6 6 6 6	300 3 37.8 C1 37.8 C1 31.8 C1 54.7 C1 22.6 C1 88.6 C1 53.7 C1 44.3 C1 45.3	4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H15 M	Counts 45 0 45 0 45 0 45 0 45 Na 0	VS. Mass-to- (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)- (M+K)+ (M+K)+ (M+K)+	Charge (m/2	z)				Sum %	
4 2 0 MS Spectru m/z 270.13503 271.13807 272.14014 273.14255 292.11731 293.12003 308.08798 309.09111 310.08961 Predicted Is Isotope	m P z 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	250 eak List Abund 9193 1781 139 8 46 9 76 16 6 6 pe Matc 7	300 3 37.8 C1 37.8 C1 31.8 C1 54.7 C1 22.6 C1 88.6 C1 53.7 C1 44.3 C1 45.4	4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M 2 M 2 M	Counts 15 0 15 0 15 0 15 0 15 Na 0 15 Na 0 (N5 0 (N5 0 (N5 0) Diff (ppm)	VS. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)- (M+K)+ (M+K)+ (M+K)+ (M+K)+	Charge (m/2	z) d %	Abund Sum %		50 Calc Abund	and the second se	
4 2- 0 MS Spectru <i>m/z</i> 270.13503 271.13807 272.14014 272.14014 273.14255 292.11731 293.12003 308.08798 309.09111 308.08798 309.09111 Predicted Is Isotope 1	m P z 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 5000	250 eak List Abund 9193 1781 139 8 46 9 76 16 6 6 6 6 6 6 6 6	300 3 37.8 C1 31.8 C1 31.8 C1 54.7 C1 22.6 C1 88.6 C1 53.7 C1 44.3 C1 44.3 C1 44.3 C1 h Table Calc m 270.	ermula 4 H16 N 4 H16 N 4 H16 N 4 H16 N 4 H15 N 4 H16 N 4 H15 N 4 H1	Counts 45 0 45 0 45 0 45 0 45 Na 0 40 Na 0	VS. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)- (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+ (M+K)+	Charge (m/2	d % 100	Abund Sum %	82.66		84.11	
4 2 0 MS Spectru m/z 270.13503 271.13807 272.14014 273.14255 292.11731 293.12003 308.08798 309.09111 310.08961 Predicted Is Isotope	m P z 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2	250 eak List Abund 9193 1781 139 8 46 9 76 16 6 6 pe Matc 270.13503	300 3 Fo 37.8 C1 31.8 C1 954.7 C1 22.6 C1 88.6 C1 53.7 C1 44.3 C1 44.3 C1 44.3 C1 44.3 C1 b b b c c c c c c c c	4 H16 M 4 H16 M 4 H16 M 4 H16 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M 4 H15 M 2 M 2 M	Counts 15 0 15 0 15 0 15 0 15 Na 0 15 Na 0 (N5 0 (N5 0 (N5 0) Diff (ppm)	vs. Mass-to- Ion (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+H)+ (M+Na)- (M+Na)- (M+K)+ (Charge (m/2	z) d %	Abund Sum %			and the second se	

20. Compound 3t

¹H NMR in DMSO-d₆



Qualitative Compound Report

Sample Type Instrument M Acq Method IRM Calibrat Comment Sample Grou	Name tion S	Status Info	Success	ent 1 S_25072012.m	Position User Na Acquire DA Meti	me d Time 11/1	9/2012 2:12:25 PM				
Compound	Tab	e						MFG	Diff		
Compou		abel RT 116 N4 O3 0.171		Mass 312.12151	Formula C16 H16 N4		MFG Formula C16 H16 N4 O3		2.36	DB Formula	
Compound	Lab	el	m/z	RT	Algorithm		Mass				
and the second state of th			313.12878	0.171	Find by Molecular Feature						
8- 6- 4-			313.12878 C16 H17 N4 O3	HJC11 1	5						
2		200 2	50 300 35	0 400 450	500 550	600 650 700	750 800 850	900 95	0		
0	150			Counts	vs. Mass-to-0	∠narge (m/z)					
0		ank Lict									
0 MS Spectru	m P		Formula		Ion						
0 MS Spectru m/z	m P	Abund	Formula		Ion (M+H)+						
0 MS Spectru <i>m/z</i> 313.12878	m P z	Abund 95061	C16 H17 N4 (03	(M+H)+						
0 MS Spectru <i>m/z</i> 313.12878 314.13235	m Po	Abund 95061 17694.3	C16 H17 N4 0 C16 H17 N4 0	03	(M+H)+ (M+H)+						
MS Spectru m/z 313.12878 314.13235 315.13432	m P z 1 1	Abund 95061 17694.3 2834.8	C16 H17 N4 C C16 H17 N4 C C16 H17 N4 C	03	(M+H)+						
MS Spectru m/z 313.12878 314.13235 315.13432 Predicted Is	m P z 1 1 1 soto	Abund 95061 17694.3 2834.8 pe Matc	C16 H17 N4 (C16 H17 N4 (C16 H17 N4 (C16 H17 N4 (h Table	03 03 03	(M+H)+ (M+H)+ (M+H)+	Calc Abund 94	Abund Sum %		alc Abund S	um 04	
MS Spectru m/z 313.12878 314.13235 315.13432 Predicted Is Isotope	m Po z 1 1 1 soto m/z	Abund 95061 17694.3 2834.8 pe Matc	C16 H17 N4 C C16 H17 N4 C C16 H17 N4 C C16 H17 N4 C h Table Calc m/z	03 03 03 Diff (ppm)	(M+H)+ (M+H)+ (M+H)+ Abund %	Calc Abund %	the second s		alc Abund S	Contraction of the second s	
MS Spectru m/z 313.12878 314.13235 315.13432 Predicted Is	m P z 1 1 1 soto m/z 3	Abund 95061 17694.3 2834.8 pe Matc	C16 H17 N4 C C16 H17 N4 C C16 H17 N4 C C16 H17 N4 C h Table Calc m/z 313.12952	03 03 03 Diff (ppm) 2.36	(M+H)+ (M+H)+ (M+H)+		6 Abund Sum % 100 9.08	82.24 15.31	alc Abund S	sum % 82.36 15.71	

References:

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