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Synthesis of Radiolabeled Biphenylsulfonamide Matrix Metalloproteinase Inhibitors as New Potential PET Cancer Imaging Agents

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Abstract—Novel matrix metalloproteinase (MMP) inhibitor radiotracers, (*S*)-3-methyl-2-(2′,3′,4′-methoxybiphenyl-4-sulfonylamino)-butyric acid [^{11}C]methyl ester (**1a–c**), (*S*)-3-methyl-2-(2′,3′,4′-fluorobiphenyl-4-sulfonylamino)-butyric acid [^{11}C]methyl ester (**1d–f**), and (*S*)-3-methyl-2-(4′-nitrobiphenyl-4-sulfonylamino)-butyric acid [^{11}C]methyl ester (**1g**), a series of substituted biphenylsulfonamide derivatives, have been synthesized for evaluation as new potential positron emission tomography (PET) cancer imaging agents.

Matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that have been shown to play a significant physiological role in the degradation and remodeling of connective tissues.[1] Several MMPs are expressed in cancers at much higher levels than in normal tissue and the extent of expression has been implicated in tumor growth, stage, invasiveness, metastasis and angiogenesis in both human and animal cancers.[2–4] The overexpression of MMPs in cancers provides a potential target for tumor imaging by biomedical imaging technique positron emission tomography (PET).[5,6] MMP inhibitors (MMPIs) can significantly reduce the growth rate of both primary and secondary tumors and can block the process of metastasis.[7] MMPIs currently undergoing clinical trails as therapeutic agents are represented by two general chemical classes, namely succinate-type structures, exemplified by Batimastat, Marimastat and RO 32-3555, and sulfonamides, including CGS 27023A, AG-3340 and BAY-12-9566 (Fig. 1).[8–16] MMPI radiotracers labeled with carbon-11[17–20] or fluorine-18[21,22] may enable non-invasive monitoring of cancer MMP levels and cancer response to MMPI therapy using PET imaging techniques.

In our previous work,[17–20] CGS 27023A was chosen as the parent compound for the design and synthesis of radiolabeled MMPIs, and the carbon-11 labeling was focused on the labeling both at the aminohydroxyl position of hydroxamic acid CGS 27023A to prepare [^{11}C]methylated CGS 27023A analogues, and at the methoxyphenyl position of hydroxamic acid CGS 25966,[23,24] an analogue of CGS 27023A, to prepare

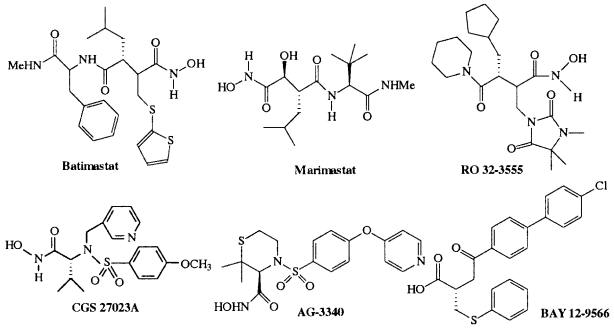

Figure 1. Representative MMP inhibitors.

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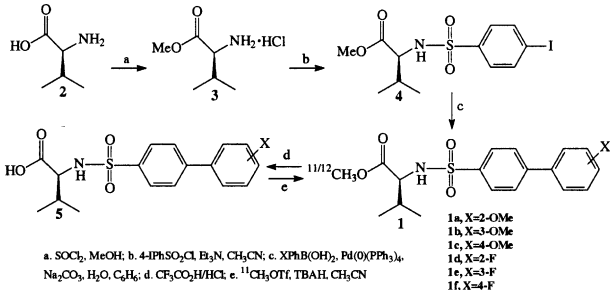
[^{11}C]CGS 25966. In this ongoing study, we chose biphenylsulfonamide MMPIs[25] as the target molecules and labeled these compounds with carbon-11 at the methyl ester moiety to make their [^{11}C]methyl esters. Here, we report the synthesis of (*S*)-3-methyl-2-(X-methoxybiphenyl-4-sulfonylamino)-butyric acid [^{11}C]methyl ester (X = 2′, **1a**; X = 3′, **1b**; X = 4′, **1c**), (*S*)-3-methyl-2-(X-fluorobiphenyl-4-sulfonylamino)-butyric acid [^{11}C]methyl ester (X = 2′, **1d**; X = 3′, **1e**; X = 4′, **1f**), and (*S*)-3-methyl-2-(4′-nitrobiphenyl-4-sulfonylamino)-butyric acid [^{11}C]methyl ester (**1g**) (Schemes 1 and 2).

The synthesis of **1a–f** was presented in Scheme 1 (method A). The commercially available starting material amino acid L-valine (**2**) was converted into its ester L-valine methyl ester hydrochloride (**3**). The ester **3** was coupled with 4-iodophenylsulfonyl chloride in the presence of triethylamine to give 4-iodobenzenesulfonamide intermediate (*S*)-3-methyl-2-(4-iodobenzenesulfonylamino)-butyric acid methyl ester (**4**). Utilizing Suzuki reaction,[26] iodide **4** was coupled with a variety of methoxyl- and fluoro-substituted benzeneboronic acid via palladium catalysis in refluxing benzene to yield substituted biphenylsulfonamide derivatives, (*S*)-3-methyl-2-(X-methoxybiphenyl-4-sulfonylamino)-butyric acid methyl ester (X = 2′, **1a**; X = 3′, **1b**; X = 4′, **1c**) and (*S*)-3-methyl-2-(X-fluorobiphenyl-4-sulfonylamino)-butyric acid methyl ester (X = 2′, **1d**; X = 3′, **1e**; X = 4′, **1f**), as the unlabeled standard samples. Hydrolysis of the methyl esters (**1a–f**) using trifluoroacetic acid in the presence of concentrated hydrochloric acid gave the corresponding carboxylic acid derivatives, (*S*)-3-methyl-2-(X-methoxybiphenyl-4-sulfonylamino)-butyric acid (X = 2′, **5a**; X = 3′, **5b**; X = 4′, **5c**) and (*S*)-3-methyl-2-(X-fluorobiphenyl-4-sulfonylamino)-butyric acid (X = 2′, **5d**; X = 3′, **5e**; X = 4′, **5f**), as the precursors for radiolabeling.

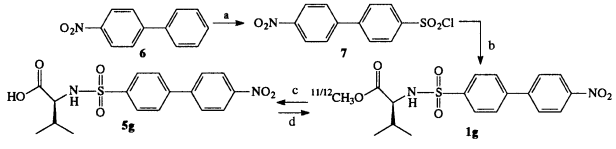
Alternatively, **1g** was prepared from appropriately substituted biphenyl starting material 4-nitrobiphenyl (**6**) as shown in Scheme 2 (method B). Commercially available **6** was reacted with chlorosulfonic acid in chloroform to give an intermediate 4-nitrobiphenylsulfonic acid without further purification, which was converted to the corresponding 4′-nitrobiphenyl-4-sulfonyl chloride (**7**) in refluxing thionyl chloride. The sulfonyl chloride **7** was coupled with amino acid ester **3** in the presence of triethylamine to give unlabeled standard sample (*S*)-3-methyl-2-(4′-nitrobiphenyl-4-sulfonylamino)-butyric acid methyl ester (**1g**). The ester **1g** required trifluoroacetic acid hydrolysis to the precursor (*S*)-3-methyl-2-(4′-nitrobiphenyl-4-sulfonylamino)-butyric acid (**5g**) as previously described.



Scheme 1. Synthesis of **1a–f** (method A).

The carboxylates (**5a–g**) were alkylated under basic conditions using tetrabutylammonium hydroxide (TBAH) with [^{11}C]methyl triflate[27,28] through ^{11}C-*O*-methylation method[29–32] and isolated by solid-phase extraction (SPE) purification[29,30] to produce pure target compounds (**1a–g**) in 40–60% radiochemical yields (based on $^{11}CO_2$, decay corrected to end of bombardment), in 20–25 min synthesis time.[33] The large polarity difference between the acid precursor and the labeled *O*-methylated product permitted the use of SPE technique for purification of radiotracer from radiolabeling reaction mixture. The reaction mixture was diluted with $NaHCO_3$ and loaded onto C-18 cartridge by gas pressure. The cartridge column was washed with water to remove unreacted acid precursor, non-reacted [^{11}C]methyl triflate and reaction solvent acetonitrile, and then final labeled product was eluted with ethanol. Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC methods, which employed a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 mm × 250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 1.5 mL/min flow rate, and UV (240 nm) and γ-ray (NaI) flow detectors. Retention times in this HPLC system were: RT**5a** = 2.23 min, RT**5b** = 2.25 min, RT**5c** = 2.14 min, RT**5d** = 2.26 min, RT**5e** = 2.32 min, RT**5f** = 2.40 min, RT**5g** = 2.06 min; RT**1a** = 5.18 min, RT**1b** = 4.85 min, RT**1c** = 4.31 min, RT**1d** = 4.69 min, RT**1e** = 5.14 min, RT**1f** = 5.12 min, RT**1g** = 3.71 min. The chemical purity of precursors **5a–g**, and standard samples **1a–g** was >95%, radiochemical purity of target radiotracers **1a–g** was >99%, and the chemical purity of target radiotracers **1a–g** was >95%. The average (n = 5–8) specific activity of target radiotracers **1a–g** was 0.6–0.8 Ci/μmol at end-of-synthesis (EOS).

The stereochemistry of radiolabeled biphenylsulfonamide analogues prepared here is different from the stereochemistry of carbon-11 CGS 27023A analogues prepared in our previous works[17–20] and fluorine-18 MMPIs prepared by Furumoto et al.[21,22] Compounds **1a–g** was prepared starting from L-valine, which is



Scheme 2. Synthesis of **1g** (method B).

stereochemical *S*-form, and they are stereochemical *S*-forms.[25] Previously reported radiolabeled MMPIs[17–22] were prepared starting from D-valine, which is stereochemical *R*-form, and they are stereochemical *R*-forms. The starting stereochemistry in this report is defined by the starting material L-valine. Based on the organic reaction mechanism and stereochemistry theory, the racemization of a chiral compound is due to the conversion to its non-chiral analogue. In the synthetic approaches shown in Scheme 1 and 2, there is not any formation of non-chiral intermediates, and the chiral center is not destroyed by any synthetic step. Therefore, we can assume that stereochemistry is conserved throughout the synthetic sequences and no racemization occurred during the various steps.

Compounds **4**, **5** and **1** have analytical data such as mp, ^{1}H NMR and MS in agreement with the indicated structures.[34]

Compound **5c** was chosen as the parent compound, since it is a potent MMP inhibitor for several MMP subtypes such as MMP-1 (IC_{50}, 1.5 μM), MMP-2 (IC_{50}, 0.003 μM), MMP-3 (IC_{50}, 0.008 μM), MMP-7 (IC_{50}, 7.2 μM), MMP-9 (IC_{50}, 2.2 μM), and MMP-13 (IC_{50}, 0.006 μM), and compounds **5f** and **5g** have similar activities for these MMP subtypes.[25] In order to examine relative inhibition effects of the modified compounds methyl esters (**1a–g**) in comparison with the parent compound **5c** and reference compounds **5f** and **5g** on MMP activity, a fibril degradation assay[17,18,35] was performed using fluorogenic substrates specific to MMP-13. MMP-13 (collagenase) specific fluorogenic substrates (Molecular Probe) (12.5 μM) and *p*-aminophenylmercuric acetate (APMA) activated human MMP-13 (Calbiochem) (100 ng) were incubated at room temperature for 2 h in the presence of individual modified compound or parent compound or reference compounds (20 nM in DMSO) in a reaction mixture (total volume of 100 μL) consisting of Tris–HCl (500 mM, pH 7.6), NaCl (1.5 M), $CaCl_2$ (50 mM) and sodium azide (2 mM). Sodium azide is a stabilizer for MMP enzyme and substrate and a potent quencher for singlet oxygen, which led to the biosynthesis of MMP enzyme and substrate, and the changes of the structures. Based on the chemical structures of **1a–g**, **5c**, **5f** and **5g**, the methyl ester analogues **1a–g** are stable as the carboxylic acid analogues **5c**, **5f** and **5g** in the media at room temperature throughout the entire time of the assay. The esters would not hydrolyze in the media, and the activity observed is due to the esters not the acids. Digestion of the intensively fluorophore labeled substrate by MMP-13 would liberate fluorophores from a quenching effect of nearby fluorophores. Fluorescent intensity was measured by FluoroMax-2 spectrofluorometer (Instruments S.A., Inc.). The absorption wavelength and the emission wavelength were set to 382 and 441 nm, respectively. A Student's *t*-test was conducted to examine the statistical significance of the inhibition effects, and *p* values less than 0.05 were considered statistically significant, in three experiments. An assay without presence of any to be tested compound or parent compound but distilled water was set as a negative control. The inhibition levels are normalized with the negative control, which the distilled water was used to replace tested inhibiting substance parent compound **5c** or any individual compound **1a–g**, **5f** or **5g**. Inhibition Effect (IE) = 1 − Relative Activity; Relative Activity = Tested Inhibiting Substance/Negative Control. It was assumed that Relative Activity for Negative Control = 1, then Inhibition Effect of Negative Control = 0. The results show that all these modified compounds **1a–g** (IE 0.9419–0.8825) exhibit strong inhibitory effectiveness on MMP-13 ($p < 0.01$) with a slight variation at a level greater than or equal to or similar to the parent compound **5c** (IE 0.9380), at the concentration of 20 nM (Table 1).

A molecule to be an effective MMP inhibitor requires a functional group (e.g., carboxylic acid, hydroxamic acid, and sulfhydryl, etc.) capable of chelating the active-site zinc (II) ion; this group is called zinc binding group or ZBG.[16] A MMP inhibitor molecule has at least one functional group, which provides a hydrogen bond interaction with the enzyme backbone; and one or more side chains, which undergo effective van der Waals interactions with the enzyme subsites. It is now apparent that this requirement can be satisfied by a variety of different structural classes of MMP inhibitors (MMPIs), which have been discovered and developed by a number of methods including structure-based design and synthesis and combinatorial chemistry.[16] Therefore, it was assumed that the structural modification of carboxylic acid derivatives **5a–g** to methylated ester derivatives **1a–g**, is not likely to cause a major change in their inhibitory properties, because the ZBG of *O*-methylated carboxylic acid has the same function as the ZBG of carboxylic acid to coordinate with zinc at the active site. Moreover, the methylation modification may increase their biological activities, in which in vivo biodistribution study of methyl ester prodrug showed a better uptake in tumor and higher tumor/organ uptake ratios in comparison with its acid drug.[22] The in vitro biological assay of the modified compounds (**1a–g**) in comparison with the parent compound **5c** also provides the evidence to support this assumption as indicated in Table 1.

Our idea to make carbon-11 labeled methyl ester prodrug, rather than carbon-11 labeled acid drug, was

Table 1. Inhibition effects of biphenylsulfonamide MMP inhibitors on MMP-13 activity

Compd (concn 20 nM)	Inhibition effect ($p < 0.01$)
Negative control	0
5c	0.9380 ± 0.014[a]
1a	0.9419 ± 0.014[a]
1b	0.9389 ± 0.009[a]
1c	0.9353 ± 0.015[a]
5f	0.9180 ± 0.017[a]
1d	0.9225 ± 0.009[a]
1e	0.9194 ± 0.012[a]
1f	0.9102 ± 0.011[a]
5g	0.8967 ± 0.021[a]
1g	0.8825 ± 0.023[a]

[a]It indicated the standard deviation was in three experiments and the inhibition effect was significant different from negative control based on the Student's *t*-test ($p < 0.01$).

based on that (1) it is easy to make the methyl ester prodrug, and it is hard to make the acid drug, from the radiochemistry point; (2) the carboxylate ester is equivalent to carboxylic acid to bind zinc in structure–activity relationship (SAR) as stated in the literature;[16] and (3) the methyl ester prodrug showed a better uptake in tumor and higher tumor/organ uptake ratios than its acid drug in the in vivo biodistribution studies as indicated in the literature,[22] which has been recently published as a full paper by Furumoto et al.,[36] so more details are known about the study. Radio-thin-layer-chromatographic (radio-TLC) analysis of radiolabeled methyl ester prodrug metabolites revealed that administered methyl ester prodrug was easily converted to the parent acid drug in vivo and accumulated in tumor tissue. Therefore, it was concluded radiolabeled methyl ester is suitable as the prodrug of radiolabeled acid with potent efficacy. A reviewer of this work criticized that it is still important to demonstrate the stability of the methyl ester compounds in the in vitro assay media, since the reviewer concerned that though azide is present in the assay to stabilize the enzyme and the substrate, it is also a potent nucleophile and could catalyze ester hydrolysis. We feel in the buffer media consisting of Tris–HCl (500 mM, pH 7.6) at room temperature, the conditions are moderate and near neutral, azide should be present as a stabilizer first, although it is a nucleophile, and methyl ester should be stable in these conditions. Following the reviewer's suggestion, we used HPLC methods[37] to monitor the behavior of the methyl esters in the assay media, which two different HPLC systems (acid **5c** in the media and methyl ester **1c** in the media) were employed. The results showed the methyl esters are not converted to the acids or any other derivative in the assay media in light of their retention times aforementioned in the HPLC chromatograms. We concluded that the methyl esters are stable and would not hydrolyze to acids in the in vitro inhibition assay as indicated by HPLC analysis; the methyl esters have similar inhibition of enzyme activity in comparison with the acids; and the methyl ester prodrug was converted to the acid drug in the in vivo metabolites analysis as shown by radio-TLC.[36]

In summary, the synthetic procedures that provide new biphenylsulfonamide MMP inhibitor radiotracers **1a–g** have been developed. Preliminary findings from in vitro biological assay indicate the synthesized analogues have strong inhibitory effectiveness on MMP-13 in comparison with the parent compound **5c**. These results warrant further evaluation of these radiotracers as new potential PET cancer imaging agents in vivo.

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33. *Typical experimental procedure for the radiosynthesis of [^{11}C]* **1a**–**g**. Acid precursor (**5a**–**g**) (0.6–1.0 mg) was dissolved in CH_3CN (300 μL). To this solution was added tetrabutylammonium hydroxide (TBAH) (2–3 μL, 1 M solution in methanol). The mixture was transferred to a small volume, three-neck reaction tube. [^{11}C]methyl triflate was passed into the air-cooled reaction tube at −15 to −20 °C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until activity reached a maximum (~3 min), then the reaction tube was heated at 70–80 °C for 3 min. The contents of the reaction tube were diluted with 0.1 M $NaHCO_3$ (1 mL). This solution was passed onto a semi-prep C-18 silica guard cartridge column 1 × 1 cm, which was obtained from E. S. Industries, Berlin, NJ, USA, and part number 300121-C18-BD 10 μ, by gas pressure. The cartridge was washed with H_2O (2 × 3 mL), and the aqueous washing was discarded. The product was eluted from the column with EtOH (2 × 3 mL), and then passed onto a rotatory evaporator. The solvent was removed by evaporation under high vacuum. The labeled product (**1a**–**g**) was formulated with 50 mM NaH_2PO_4, whose volume was dependent upon the use of the labeled product (**1a**–**g**) in tissue biodistribution studies (~6 mL, 3 × 2 mL) or in micro-PET imaging studies (1–3 mL) of the breast cancer athymic mice, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis time was ~20 min. The decay corrected yield, from $^{11}CO_2$, was 40–60%.

34. **4**, white solid, 97% yield, mp 100–101 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86–0.88 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.95–0.97 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 2.01–2.10 (m, 1H, $(CH_3)_2\underline{CH}$), 3.48 (s, 3H, $CO_2\underline{CH_3}$), 3.71–3.76 (dd, J = 5.1, 9.6 Hz, 1H, CHN), 5.14–5.18 (d, J = 10.3 Hz, 1H, NH), 7.53–7.55 (d, J = 8.1 Hz, 2H, H-Ph), 7.84–7.87 (d, J = 8.1 Hz, 2H, H-Ph). **5a**, a gray solid, 98% yield, mp 178–179 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.79–0.81 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.82–0.84 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.90–2.02 (m, 1H, $(CH_3)_2\underline{CH}$), 3.53–3.58 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 3.78 (s, 3H, CH_3OPh), 5.11–5.15 (d, J = 10.3 Hz, 1H, NH), 7.02–7.07 (t, J = 8.1 Hz, 1H, H-PhOMe), 7.12–7.14 (d, J = 8.1 Hz, 1H, H-PhOMe), 7.30–7.33 (d, J = 7.4 Hz, 1H, H-PhOMe), 7.36–7.41 (t, J = 7.4 Hz, 1H, H-PhOMe), 7.61–7.65 (d, J = 8.1 Hz, 2H, H-Ph), 7.76–7.79 (d, J = 8.1 Hz, 2H, H-Ph). LR/MS (CI, CH_4, m/z): 363.1 (M^+, 29%), 247.0 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{18}H_{21}NO_5S$ 363.1140, found 363.1157. **5b**, a gray solid, 99% yield, mp 156–157 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.79–0.81 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.82–0.84 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.88–1.99 (m, 1H, $(CH_3)_2\underline{CH}$), 3.52–3.58 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 3.83 (s, 3H, CH_3OPh), 5.21–5.25 (d, J = 10.3 Hz, 1H, NH), 6.98–7.01 (d, J = 8.1 Hz, 1H, H-PhOMe), 7.25 (s, 1H, H-PhOMe), 7.27–7.30 (d, J = 8.1 Hz, 1H, H-PhOMe), 7.38–7.43 (t, J = 8.1 Hz, 1H, H-PhOMe), 7.81–7.84 (d, J = 8.8 Hz, 2H, H-Ph), 8.07–8.10 (d, J = 8.8 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 363.1 (M^+, 25%), 183.1 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{18}H_{21}NO_5S$ 363.1140, found 363.1141. **5c**, a gray solid, 99% yield, mp 169–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.78–0.80 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.82–0.84 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.88–2.00 (m, 1H, $(CH_3)_2\underline{CH}$), 3.51–3.55 (d, J = 5.1, 9.7 Hz, 1H, CHN), 3.80 (s, 3H, CH_3OPh), 5.14–5.18 (d, J = 10.3 Hz, 1H, NH), 7.03–7.06 (d, J = 8.1 Hz, 2H, H-Ph), 7.67–7.70 (d, J = 8.1 Hz, 2H, H-Ph), 7.76–7.78 (d, J = 9.5 Hz, 1H, H-Ph), 7.79 (s, 2H, H-Ph), 8.01–8.05 (d, J = 9.5 Hz, 1H, H-Ph). **5d**, a gray solid, 93% yield, mp 141–143 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98–1.00 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.02–1.03 (d, J = 6.6 Hz, 3H, $(CH_3)_2\underline{CH}$), 2.10–2.20 (m, 1H, $(CH_3)_2\underline{CH}$), 3.74–3.79 (dd, J = 6.0, 9.5 Hz, 1H, CHN), 5.07–5.11 (d, J = 10.3 Hz, 1H, NH), 7.51–7.58 (m, 2H, H-FPh), 7.66–7.68 (m, 1H, H-FPh), 7.74–7.79 (t, J = 8.1 Hz, 1H, H-FPh), 7.91–7.93 (d, J = 8.1 Hz, 2H, H-Ph), 8.04–8.07 (d, J = 8.1 Hz, H-Ph). LRMS (CI, CH_4, m/z): 351.1 (M^+, 2.2%), 171.0 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{17}H_{18}FNO_4S$ 351.0941, found 351.0934. **5e**, a gray solid, 97% yield, mp 147–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98–1.00 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.01–1.03 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 2.02–2.20 (m, 1H, $(CH_3)_2\underline{CH}$), 3.72–3.77 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 5.10–5.13 (d, J = 10.3 Hz, 1H, NH), 7.43–7.48 (t, J = 7.4 Hz, 1H, H-FPh), 7.72–7.85 (m, 3H, H-FPh), 8.02–8.11 (dd, J = 8.1 Hz, 3H, H-Ph), 9.29–8.32 (d, J = 8.8 Hz, 1H, H-Ph). LRMS (CI, CH_4, m/z): 351.1 (M^+, 1%), 171.0 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{17}H_{18}FNO_4S$ 351.0941, found 351.0938. **5f**, a gray solid, 97% yield, mp 161–163 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.78–0.80 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.82–0.84 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.90–1.98 (m, 1H, $(CH_3)_2\underline{CH}$), 3.52–3.57 (dd, J = 5.9, 9.6 Hz, 1H, CHN), 5.13–5.16 (d, J = 10.3 Hz, 1H, NH), 7.30–7.36 (t, J = 8.8 Hz, 2H, H-FPh), 7.43–7.48 (t, J = 8.8 Hz, 2H, H-FPh), 7.76–7.81 (m, 3H, H-Ph), 8.07–8.10 (d, J = 9.6 Hz, 1H, H-Ph). **5g**, a yellow solid, 99% yield, mp 147–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.79–0.81 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.82–0.84 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.90–1.98 (m, 1H, $(CH_3)_2\underline{CH}$), 3.54–3.59 (dd, J = 5.3. 10.3 Hz, 1H, CHN), 5.73–5.76 (d, J = 9.7 Hz, 1H, NH), 7.88–7.7.91 (d, J = 8.8 Hz, 2H, H-Ph), 7.91–7.96 (d, J = 8.8 Hz, 2H, H-Ph), 8.00–8.03 (d, J = 8.8 Hz, 2H, H-Ph), 8.32–8.35 (d, J = 8.8 Hz, 2H, H-Ph). **1a**, a white solid, 88% yield, mp 78–79 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90–0.92 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.97–0.99 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.99–2.11 (m, 1H, $(CH_3)_2\underline{CH}$), 3.43 (s, 3H, CO_2CH_3), 3.76–3.79 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 3.86 (s, 3H, CH_3OPh), 5.10–5.13 (d, J = 10.3 Hz, 1H, NH), 6.99–7.02 (d, J = 8.8 Hz, 1H, H-Ph), 7.03–7.08 (dd, J = 8.1, 8.8 Hz, 1H, H-Ph), 7.28–7.31 (m, 2H, H-Ph), 7.63–7.66 (d, J = 8.8 Hz, 2H, H-Ph), 7.82–7.85 (d, J = 8.8 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 377.1 (M^+, 17%), 318.1 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{19}H_{23}NO_5S$ 377.1297, found 377.1303. **1b**, a white solid, 89% yield, mp 93–95 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88–0.90 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.96–0.98 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.99–2.00 (m, 1H, $(CH_3)_2\underline{CH}$), 3.43 (s, 3H, CO_2CH_3), 3.76–3.81 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 3.88 (s, 3H, CH_3OPh), 5.12–5.15 (d, J = 10.1 Hz, 1H, NH), 6.95–6.98 (dd, J = 1.8, 8.4 Hz, 1H, H-PhOMe), 7.11–7.12 (d, J = 1.6 Hz, 1H, H-PhOMe), 7.37–7.42 (t, J = 8.1 Hz, 2H, H-PhOMe), 7.68–7.71 (d, J = 8.8 Hz, 2H, H-Ph), 7.87–7.90 (d, J = 8.8 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 377.1 (M^+, 15%), 318.0 (100%). HRMS (CI, CH_4, m/z): calcd for

$C_{19}H_{23}NO_5S$ 377.1297, found 377.1302. **1c**, a white solid, 93% yield, mp 156–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88–0.90 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.96–0.98 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 2.02–2.11 (m, 1H, $(CH_3)_2\underline{CH}$), 3.42 (s, 3H, CO_2CH_3), 3.75–3.80 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 3.87 (s, 3H, CH_3OPh), 5.11–5.15 (d, J = 9.7 Hz, 1H, NH), 6.99–7.02 (d, J = 8.1 Hz, 2H, H-Ph), 7.54–7.57 (d, J = 8.8 Hz, 2H, H-Ph), 7.65–7.67 (d, J = 8.1 Hz, 2H, H-Ph), 7.84–7.87 (d, J = 8.1 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 377.1 (M^+, 28%), 183.1 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{19}H_{23}NO_5S$ 377.1297, found 377.1308. **1d**, a white solid, 94% yield, mp 82–83 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89–0.91 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.97–0.99 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 1.98–2.12 (m, 1H, $(CH_3)_2\underline{CH}$), 3.43 (s, 3H, CO_2CH_3), 3.76–3.81 (dd, J = 5.1, 10.1 Hz, 1H, CHN), 5.13–5.16 (d, J = 10.1 Hz, NH), 7.15–7.36 (m, 2H, H-FPh), 7.38–7.46 (m, 2H, H-FPh), 7.66–7.68 (d, J = 8.1 Hz, 2H, H-Ph), 7.88–7.91 (d, J = 8.1 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 366.1 ($M^+ + 1$, 1%), 171.1 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{18}H_{20}FNO_4S$ 365.1097, found 365.1173. **1e**, a white solid, 87% yield, mp 106–107 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88–0.90 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.97–0.99 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 2.01–2.10 (m, 1H, $(CH_3)_2\underline{CH}$), 3.44 (s, 3H, CO_2CH_3), 3.77–3.82 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 5.18–5.21 (d, J = 10.3 Hz, 1H, NH), 7.05–7.16 (t, J = 8.1 Hz, 1H, H-FPh), 7.27–7.32 (dt, J = 2.2, 9.6 Hz, 1H, H-FPh), 7.37–7.46 (m, 2H, H-FPh), 7.67–7.70 (d, J = 8.8 Hz, 2H, H-Ph), 7.89–7.92 (d, J = 8.8 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 365.1 (M^+, 1%), 171.1 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{18}H_{20}FNO_4S$ 365.1097, found 365.1097. **1f**, a white solid, 97% yield, mp 127–128 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88–0.90 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 0.97–0.99 (d, J = 6.6 Hz, 3H, $\underline{CH_3}CH$), 2.01–2.11 (m, 1H, $(CH_3)_2\underline{CH}$), 3.44 (s, 3H, CO_2CH_3), 3.76–3.82 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 5.09–5.13 (d, J = 10.3 Hz, 1H, NH), 7.57–7.20 (t, J = 8.8 Hz, 2H, H-FPh), 7.55–7.59 (m, 2H, H-FPh), 7.64–7.67 (d, J = 8.1 Hz, 2H, H-Ph), 7.87–7.90 (d, J = 8.8 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 365.1 (M^+, 3.3%), 171.1 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{18}H_{20}FNO_4S$ 365.1097, found 365.1078. **1g**, a light yellow solid, 95% yield, mp 156–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89–0.91 (d, J = 7.3 Hz, 3H, $\underline{CH_3}CH$), 0.97–1.00 (d, J = 7.3 Hz, 3H, $\underline{CH_3}CH$), 2.04–2.14 (m, 1H, $(CH_3)_2\underline{CH}$), 3.48 (s, 3H, CO_2CH_3), 3.80–3.85 (dd, J = 5.1, 10.3 Hz, 1H, CHN), 5.21–5.24 (d, J = 10.3 Hz, 1H, NH), 7.74–7.78 (m, 4H, H-Ph), 7.95–7.98 (d, J = 8.1 Hz, 2H, H-Ph), 8.33–8.36 (d, J = 8.8 Hz, 2H, H-Ph). LRMS (CI, CH_4, m/z): 393.0 (M^+, 0.3%), 333.1 (100%). HRMS (CI, CH_4, m/z): calcd for $C_{16}H_{17}N_2O_4S$ ($M^+ - CO_2CH_3$) 333.0908, found 333.0920.

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