Graphical Abstract

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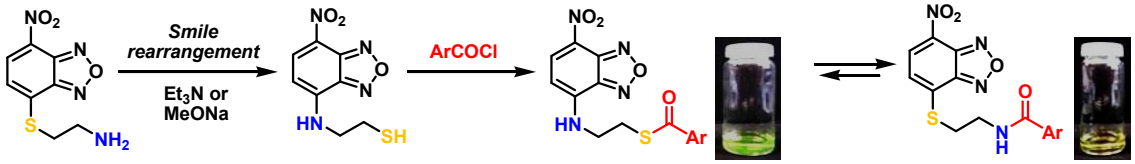

Tetrahedron Letters
journal homepage: www.elsevier.com

Studies on the acylation of 4-(2-aminoethylthio)-7-nitrobenzofurazan: the role of bases in promoting the formation of fluorescent S-acyl derivatives through S-N Smiles rearrangement

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
S-N acylation
Smiles rearrangement
Fluorescent probe
Benzofurazan
Cysteamine

ABSTRACT

The acylation of 4-(2-aminoethylthio)-7-nitrobenzofurazan has been investigated. Depending on the use of the base, a competitive Smiles rearrangement occurs during the acylation step leading to the formation of N-acyl and/or fluorescent S-acyl derivatives. The acylating agent also affects the ratio of N/S acylated isomers.

Benzofurazans (Bf) represent an interesting class of organic compounds which is receiving increasing attention due to their use in different fields of chemistry.[1] Compounds containing a benzofurazan (benzo[1,2-c][1,2,5]oxadiazole) skeleton have been widely used in the field of bioscience as fluorogenic and fluorescent reagents due to their chemical and physical properties such as the large Φ_f values of the resultant derivatives, the high reactivity to analytes and the long excitation and emission wavelengths.[2] In particular, the 4,7-disubstituted-benzofurazans found a broad application in bioanalytical chemistry[3-5] due to their unique character regarding fluorescent properties.[6] Experimental and theoretical works showed that the key factors in control of the fluorescence of 4,7-substituted benzofurazan dyes are the dipole moment across the benzene ring (from position 4 to 7) and the electron density found on the ring.[7] The commercially available 4-chloro-7-nitro-benzofurazan (NDBf-Cl) **1** (Scheme 1) and, more in general, the 7-nitro-4-substituted benzofurazans (NDBf), are 10π electron heteroaromatic substrates which exhibit an extremely high electrophilic character[8] and have been used as reactivity probes and as fluorescent labelling reagents in the study of a number of proteins.[9] In addition, the NDBf derivatives also proved to possess several biological properties finding growing application in medicinal chemistry as antileukemic/antiapoptotic agents and monoamino oxidase inhibitors.[10-11]

In the course of our studies on antiviral compounds[12-13] we became interested in the synthesis of novel hits containing the NDBf nucleus. In particular we focused on the synthesis of NDBf derivatives with general structure **A** (Scheme 1) containing an acylated cysteamine chain at C-4. Surprisingly we found that acylation of cysteamine derivative **2** led in the presence of Et_3N to the formation of desired compound **3** together with a fluorescent side product which, after NMR and MS analyses, revealed to be the S-acyl-derivative **4** (Scheme 1). The formation of this latter compound was assumed to be due to a S-N Smiles rearrangement of the cysteamine chain of **2** on the electrophilic benzofurazan core. The Smiles rearrangement is an intramolecular aromatic nucleophilic substitution of an appropriately placed nucleophile onto aromatic rings and it is usually catalysed by bases, acids or heat.[16] Few examples of O-O and S-O Smiles rearrangements on furazan, benzofurazan and benzofurazan-*N*-oxide heterocycles have been described in literature and generally they occur in the presence of strong bases such NaOH or KOMe.[14] However, to the best of our knowledge, no examples of S-N Smiles rearrangement on benzofurazans have been reported so far.

Herein, we decided to investigate the influence of the Smiles rearrangement in the acylation of the 7-(2-aminoethylthio)-4-nitrobenzofurazan. In particular, we demonstrated that the occurrence of the S-N Smiles

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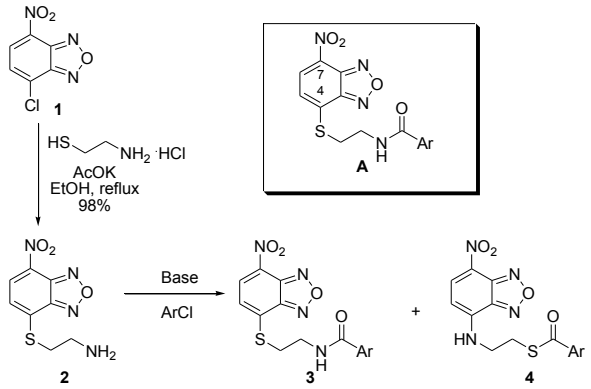
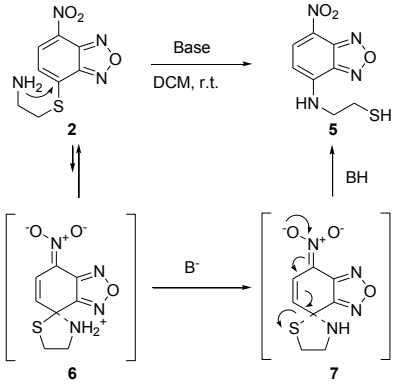
rearrangement on benzofurazan **2** is strictly dependent on the base and acylating agents used and that an appropriate setting up of the reaction conditions and a careful use of reagents could allow the selective S- or N- acylation of **2**.



Scheme 1. Acylation of cysteamine derivative **2**

Cysteamine-NDBf **2** was prepared in almost quantitative yield through the reaction of cysteamine hydrochloride (1.1 eq) with NDBf-Cl **1** (1.0 eq) in refluxing ethanol and in the presence of a catalytic amount of KOAc (Scheme 1).[10-18] As expected the more nucleophilic sulfur of cysteamine reacts with NDBf-Cl **1** faster than nitrogen atom leading selectively to C4-S derivative **2** while no traces of the C4-N regioisomer were observed. We first decided to react **2** with different bases under mild conditions (DCM, 25 °C), in order to investigate its tendency to give the S-N-Smile rearrangement (Scheme 2, Table 1). Treatment of NDBf **2** with 1-2 eq of pyridine did not lead to the formation of the rearranged NDBf **5** also after several hours and starting **2** was completely recovered from the reaction mixture (*entries 1-2*). On the other hand, when **2** was treated with the more basic Et_3N (1 eq) partial conversion into **5** was observed by ^{1}H-NMR (*entry 3*). Complete conversion was achieved by treatment of **2** with 2 eq of Et_3N (*entry 4*). The ^{1}H-NMR of compound **2** shows a typical H-5 peak at 7.74 ppm while the H-5 proton of 4-N-analogue **5** falls at 6.35 ppm. Similarly, treatment of **2** with MeONa (2 eq) led to complete conversion into **5** (*entry 5*), while in the presence of K_2CO_3 or KOH no rearrangement was observed even if higher amounts of bases were used (*entries 6-7*). According to literature data[14] the Smiles rearrangement is supposed to proceed through a S_NAr at *ipso* position with the formation of a spirocyclic Meisenheimer complex intermediate **6** (Scheme 2). The first step of the reaction is the formation of the five-membered intermediate **6** due to the attack of the nucleophilic amine at the *ipso* position. Deprotonation of **6** by a base leads to intermediate **7** which is in turn converted into rearranged compound **5**.[15]



Scheme 2. Smiles rearrangement: mechanism

Table 1. Base promoted Smile rearrangement on 2

Entry	Base	Amount (eq.)	Ratio (%)[a,b]	
			2	**5**
1	Pyr	1	100	N.D.[c]
2	Pyr	2	100	N.D.[c]
3	Et_3N	1	70	30
4	Et_3N	2	N.D.	100
5	MeONa	2	N.D.	100
6	KOH	2	100	N.D.[c]
7	K_2CO_3	2	100	N.D.[c]

[a]Ratios were determined by GC–MS and ^{1}H-NMR analysis of the crude mixtures. [b]Reactions were completed after 1h. [c]Not detected.

Since Et_3N and MeONa favor the Smiles rearrangement of **2** leading to the formation of **5**, it was reasonable to assume that under appropriate acylation conditions the formation of N- or S-acyl derivatives could be selectively achieved. Hence, compound **2** was reacted with different electron rich/poor acylating agents (namely thienyl, bezoyl and nicotinoyl chlorides) in the presence of different bases. Results are reported on Table 2.

Thienyl (ThCl), benzoyl and nicotinoyl chorides (NicCl) were reacted with **2** in the presence of pyridine at r.t. leading to the slow formation of acyl derivatives **3a-c** in several hours. On the other hand, the addition of DMAP accelerated dramatically the rate of the reaction leading to **3a** and **3c** in a few hours (5.5 and 3 h respectively, *entries 1 and 3*). The formation of benzoyl derivative **3b** proved to be faster and was accomplished in only 30 minutes (*entry 2*). However, in all cases, only traces of the S-acylated rearranged products **4** could be detected by ^{1}H-NMR. A similar behavior of **2** was observed when the same acylation reactions were carried using KOH as a base (data not shown). Benzofurazan **2** was then reacted with ThCl in the presence of 1.1 eq. of Et_3N affording in 1 h a mixture of **3a**/**4a** in a 1:1 ratio (*entry 4*). Compound **4a** proved to be fluorescent as shown in Figure 1. Increasing the amount of Et_3N to 1.5 eq led to the formation of **3a**/**4a** in a 7:3 ratio in only 30 minutes, while the further increase of the base to 2 eq resulted into a drop of the isomeric ratio to 1:1 (*entries 5-6*).

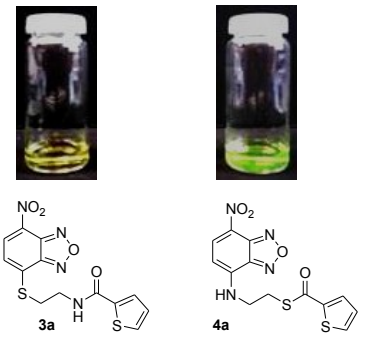
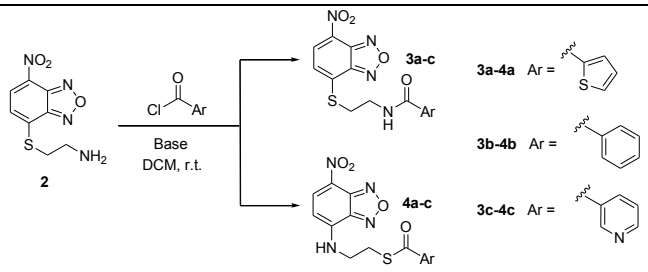


Figure 1. N-acylated and Fluorescent S-acylated derivative

Table 2. Formation of S/N-acyl derivatives 3-4 through S-N Smiles rearrangement

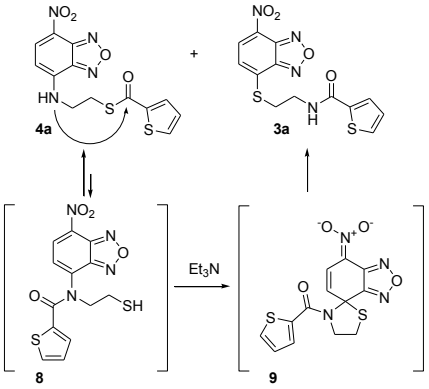


Entry	Ar	base	base (eq.)	Time (h)	Ratio (%)[a,b] 3/4
1	Thienyl	Py	2	5.5	98:2[c]
2	Ph	Py	2	0.5	97:3[c]
3	Nicotinoyl	Py	2	3	100:0[c]
4	Thienyl	Et_3N	1.1	1	55:45[c]
5	Thienyl	Et_3N	1.5	0.5	70:30
6	Thienyl	Et_3N	2	0.5	58:42
7	Thienyl	Et_3N	2	1	55/45[d]
8	Thienyl	Et_3N	2	24[e]	92/8[e]
9	Ph	Et_3N	1.1	0.5	40:60
10	Ph	Et_3N	2	0.3	27:73
11	Nicotinoyl	Et_3N	1.1	4	100:0
12	Nicotinoyl	Et_3N	2	2	100:0
13	Thienyl	NaOMe	2	24	64:36
14	Thienyl	NaOMe	2	24	35:65[d]
15	Ph	NaOMe	2	24	40:60
16	Nicotinoyl	NaOMe	2	16	100:0

[a]All the reactions were monitored by TLC analysis and stopped after consumption of the cysteamine-NDBf **2**. [b]Ratios of products were determined by ^{1}H-NMR. [c]Reaction was performed in the presence of a catalytic amount of DMAP. [d]Starting material **2** and base were premixed and the thiophene carbonyl chloride was added after 2 h. [e]The reaction was left stirring for additional 24 h after the TLC showed the consumption of **2**.

These surprising results may be explained if a competition between the acylation step of **2** and the Et_3N promoted Smiles rearrangement is assumed. Hence, it is reasonable to think that the base promotes the rearrangement of the cysteamine chain and in the meantime favors the N-acylation of **2** and the S-acylation of the forming **5**.[16] To confirm this assumption, compound **2** was treated with 2 eq of Et_3N and the reaction mixture was stirred 2 h before adding ThCl. Formation of **4a** as the only product was expected. Surprisingly, a 1:1 mixture of **3a** and **4a** was recovered from the reaction mixture (*Entry 7*). A base mediated equilibration between N- and S-acyl derivates **3a** and **4a** was then hypothesized and, in order to explain these results, we decided to treat compound **4a** with 2 eq of Et_3N. Quantitative conversion of **4a** into **3a** was observed by ^{1}H-NMR within 24h proving that compound **4a** is the kinetic product of the reaction and compound **3a** is the thermodynamic. According to Wieland and Bokelmann studies on cysteamine,[17] a plausible mechanism for the conversion of **4a** into **3a** is reported in Scheme 3. On the basis of this result we can assume that the treatment of **2** with Et_3N led to **5** and addition of the ThCl after 2h led to the formation of kinetic product **4a**. However, the base starts meanwhile to catalyze the conversion of **4a** into **3a**, thus affording a 1:1 mixture of the two products. As further corroboration, benzofurazan **2** was treated with ThCl and 2 eq of Et_3N and the reaction was left to carry out for 24h (*entry 8*) leading to the formation of **3a** as the major product in a 92:8 ratio. Interestingly, the treatment of **2** with BzCl in the presence Et_3N led to the formation of S-benzoylated **4b** as the major product in a few minutes. In particular, when 2 eq of base were used compounds **3b** and **4b** were obtained in a 27:73 ratio (*entry 10*). As shown above, benzoylation proved to be faster than thienylation and nicotinoylation. The BzCl reacts fast with the forming rearranged compound **5** and thus leads the

reaction to completion before that equilibration of **4b** into **3b** could occur. Finally, NicCl reacts with **2** in the presence of Et_3N leading to **3c** as the only product (*entries 11-12*). We then investigated the acylation of **2** in the presence of MeONa. Treatment of **2** with ThCl led to a 3:2 mixture of **3a** and **4a** in 24 h (*entry 13*). On the other hand, when compound **2** was premixed with MeONa and ThCl was added after 2 h, a 35:65 mixture of **3a** and **4a** was obtained after 24 h (*entry 14*). Even in this case an equilibration of the kinetic into the thermodynamic product occurs. However, conversion of **4a** into **3a** catalyzed by MeONa occurred slower than in the presence of Et_3N thus accounting the isolation of **4a** as the major product. Finally, the reaction of **2** with BzCl in the presence of MeONa led to compounds **3b** and **4b** in a 40:60 ratio, confirming the tendency of BzCl to favor the formation of S-acylated product. On the other hand, when NicCl was reacted with **2** only the N-nicotinoyl derivative **3c** was isolated from the reaction mixture. Due to the higher electrophilicity of its chlorocarbonyl function, it is plausible that NicCl reacts with **2** before than the base could promote the Smile rearrangement, thus accounting for the selective formation of **3c**.



Scheme 3. Equilibration of **4a** into **3a**

In conclusion, the acylation reaction of 7-(2-aminoethylthio)-4-nitrobenzofurazan **2** was thoroughly investigated. The influence of the base and of the acyl donors on the outcome of reaction as well as the tendency of the substrate **2** to give the Smiles rearrangement has been highlighted. Smiles rearrangement on NDBfs bearing a cysteamine chain at C4 occurs in the presence of Et_3N and MeONa and lead to S- or N-acylated NDBfs[18] depending on the nature of the acylating group. In some cases, the unstable S-acylated benzofurazan derivatives can be obtained as the major reaction products. Finally, the *S*-acyl derivatives **4a** and **4b** are fluorescent and represent interesting scaffolds in the development of novel bioanalytical probes for the study of protein interactions.

Acknowledgments

We gratefully acknowledge financial support provided by FP7 FLUINHIBIT project (Ref. 201634).

References and notes

1. Ioannis, M.; Takakis, P.; Hadjimihalakis, M. *J. Heterocycl. Chem.* **1990**, *27*, 177–181.
2. Lavis, L. D.; Raines, R. T. *ACS Chem. Biol*. **2008**, *3*, 142–155.
3. Bem, M.; Badea, F.; Draghici, C.; Caproiu, M. T.; Vasilescu, M.; Voicescu, M.; Beteringhe, A.; Caragheorgheopol, A.; Maganu, M.; Constantinescu, T.; Balaban, A. T. *ARKIVOC* **2007**, 87–104.
4. Numasawa, Y.; Okabe, K.; Uchiyama, S.; Santa, T.; Imai, K. *Dyes Pigm.* **2005**, *67*, 189–195.
5. Allen, G.; Lowe, G. *Biochem. J.* **1973**, *133*, 679-686.
6. a) Uchiyama, S.; Santa, T.; Fukushima, T.; Homma, H.; Imai, K. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2165; b) Uchiyama, S.; Takehira, K.; Kohtani, S.; Imai, K.; Nakagaki, R.; Tobita, S.; Santa, T. *Org. Biomol. Chem.* **2003**, *1*, 1067-1072.
7. Uchiyama, S.; Santa, T.; Imai, K. *J. Chem. Soc., Perkin Trans. 2*, **1999**, 2525–2532.
8. a) Terrier, F. in *Nucleophilic Aromatic Displacement*; Feuer, H., Ed.; VCH: New York, **1991**. b) Buncel, E.; Crampton, M. R.; Strauss, M. J.; Terrier, F. in *Electron-Deficient Aromatic and Heteroaromatic Base Interactions*; Elsevier: Amsterdam, **1984**; pp. 166 and 296. c) Terrier, F. *Chem. Rev.* **1982**, *82*, 77. d) Buncel, E.; Dust, J. M.; Terrier, F. *Chem. Rev.* **1995**, *95*, 2261.
9. a) Shipton, M.; Stuchjbury, T.; Brocklehurst, K. *Biochem. J.* **1976**, *159*, 235-244. b) Ferguson, S. T.; Lloyd, W.J.; Radda, G.K. *Biochem. J.* **1976**, *159*, 347-353. c) Uchiyama, S.; Santa, T.; Okiyama, N.; Fukushima, T.; Imai, K. *Biomed. Chromatogr.* **2001**, *15*, 295–318.
10. a) Ghosh, P. B.; Whitehouse, M. W. *J. Med. Chem.* **1968**, *11*, 1305-1311. b) Borisenko, G. G.; Kapralov, A. A.; Tyurin, V. A.; Maeda, A.; Stoyanovsky, D. A.; Kagan, V. E. *Biochemistry* **2008**, *47*, 13699-13710.
11. Fu, R. C.; Liu, X. F.; Chen, S. *J. Neurochem.* **1990**, *55*, 813–818.
12. Castagnolo, D.; Pagano, M.; Bernardini, M.; Botta, M. *Synlett* **2009**, 2093-2096.
13. a) Radi, M.; Contemori, L.; Castagnolo, D.; Spinosa, R.; Este´, J. A.; Massa, S.; Botta, M. *Org. Lett.* **2007**, *9*, 3157-3160. b) Radi, M.; Maga, G.; Alongi, M.; Angeli, L; Samuele, A.; Zanoli, S.; Bellucci, L.; Tafi, A.; Casaluce, G.; Giorgi, G.; Armand-Ugon, M.; Gonzalez, E.; Estè, J. A.; Baltzinger, M.; Bec, G.; Dumas, P.; Ennifar, E.; Botta, M. *J. Med. Chem.*. **2009**, *52*, 840–851.
14. a) Ah-Kow, G.; Terrier, F. *J. Org. Chem.* **1978**, *43*, 3578-84. b) Al-Kaysi R. O., Gallardo, I.; Guirado, G. *Molecules* **2008**, *13*, 1282-1302.
15. Attempts to isolate spirocyclic intermediate **6** resulted unsuccessful. Even in the presence of bases such DBU and DABCO (Ref. 8c) compound **6** was not isolated.
16. Warren, L. A.; Smiles, S. *J. Chem. Soc.* **1931**, 914–922.
17. Wieland, T.; Bokelmann, E. *Justus Lieb. Ann. Chem.* **1952**, *576*, 20–34.
18. Burchfield, H. P. *Nature* **1958**, *181*, 49-50
19. **General procedure for the acylation of 7-(2-aminoethylthio)-4-nitrobenzofurazan:** To a stirred solution of compound **2** (0.1 mmol) in anhydrous DCM (3 mL), the acyl chloride (0.11 mmol) followed by the appropriate base (0.2 mmol) were added. The reaction mixture was stirred at room temperature until completion. The solvent was removed in vacuo and the crude products were purified by flash column chromatography (SiO_2) using 1:1 AcOEt/hexanes, as the eluent to yield the benzofurazans **3** and **4**.
Characterization of **3a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.47 (d, 1H, *J* = 8.2 Hz), 7.67 (d, 1H, *J* = 4.3 Hz), 7.62-7.59 (m, 2H), 7.05 (t, 1H, *J* = 4.3 Hz), , 3.57 (t, 2H, *J* = 6.1 Hz), 3.49 (t, 2H, *J* = 6.1 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 143.7, 141.2, 136.5, 134.5, 133.4, 132.7, 131.7, 130.4, 128.1, 99.5, 44.6, 27.7 ppm. MS (ESI) m/z: 373 $[M+Na]^+$.
Characterization of **4a**: ^{1}H NMR (400 MHz DMSO-d_6) δ 8.46 (d, 1H, *J* = 8.3 Hz), 7.78 (d, 1H, *J* = 4.2 Hz), 7.64 (d, 1H, *J* = 4.2 Hz), 7.09 (t, 1H, *J* = 4.2 Hz), 6.70 (bs, 1H), 6.33 (d, 1H, *J* = 8.3 Hz), 3.75 (t, 2H, *J* = 6.4 Hz), 3.38 (t, 2H, *J* = 6.4 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.3, 143.4, 140.8, 136.1, 133.9, 133.2, 132.1, 131.6, 129.9, 128.1, 99.0, 44.1, 27.4 ppm. MS (ESI) m/z: 373 $[M+Na]^+$.
Characterization of **3b**: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.49 (d, 1H, *J* = 8.1 Hz), 8.19 (bs, 1H), 7.75 (d, 2H, *J* = 8.4 Hz), 7.62 (d, 1H, *J* = 8.1 Hz), 7.47-7.37 (m, 3H), 3.60 (t, 2H, *J* = 6.2 Hz), 3.51 (t, 2H, *J* = 6.2 Hz) ppm. ^{13}C NMR (100 MHz, Acetone *d-6*) δ 167.0, 153.0, 149.9, 140.3, 134.7, 131.6, 131.4, 128.3, 127.1, 122.2, 38.2, 30.3 ppm. MS (ESI) m/z: 367 $[M+Na]^+$.
Characterization of **4b**: ^{1}H NMR (400 MHz DMSO-d_6) δ 8.46 (d, 1H, *J* = 8.3 Hz), 7.84 (d, 2H, *J* = 8.5 Hz), 7.62 (t, 1H, *J* = 8.5 Hz), 7.48 (t, 2H, *J* = 8.5 Hz), 6.50 (d, 1H, *J* = 8.3 Hz), 3.68 (bs, 1H), 3.35 (t, 2H, *J* = 6.1 Hz), 3.23 (t, 2H, *J* = 6.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.4, 149, 143.4, 141.4, 137.9, 134.5, 132.6, 129.7, 127, 123.8, 95.1, 68.5, 33.3 ppm. MS (ESI) m/z: 367 $[M+Na]^+$.
Characterization of **3c**: ^{1}H NMR (400 MHz DMSO-d_6) δ 8.97 (s, 1H), 8.76 (d, 1H, *J* = 4.3 Hz), 8.45 (d, 1H, *J* = 8.1 Hz), 8.19 (d, 1H, *J* = 4.3 Hz), 7.63 (d, 1H, *J* = 8.1 Hz), 7.52 (q, 1H, *J* = 4.3 Hz), 3.38 (t, 2H, *J* = 6.1 Hz), 3.30 (t, 2H, *J* = 6.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.1, 152.9, 146.0, 145.6, 145.2, 135.7,

135.3, 129.9, 125.9, 125.3, 124.6, 117.6, 41.5, 31.7 ppm. MS (ESI) m/z: 368 $[M+Na]^+$.

20. Characterization of benzofurazan **2**: ^{1}H NMR (400 MHz, MeOD) δ 8.47 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 3.65-3.62, (m, 2H), 3.35-3.31 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.4, 142.6, 137.2, 133.5, 130.9, 122.8, 37.5, 27.7 ppm. MS (ESI) m/z: 241 $[M+H]^+$. Characterization of benzofurazan **5**: ^{1}H NMR (400 MHz, MeOD) δ 8.47 (d, *J* =8.01 Hz, 1H), 6.35 (d, *J* = 8.01 Hz, 1H), 3.65-3.62, (m, 2H), 3.35-3.31 (m, 2H). ^{13}C NMR (100 MHz, MeOD) 149.4, 142.6, 137.1, 133.5, 132.8, 130.9, 37.6, 27.8 ppm. MS (ESI) m/z: 241 $[M+H]^+$.