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Synthesis and antimicrobial activity of polyhalo isophthalonitrile derivatives

Chao Huang [a,b,†], Sheng-Jiao Yan [a,†], Neng-Qin He [a], Ya-Juan Tang [a], Xing-Hong Wang [c,*], Jun Lin [a,*]

[a] Key Laboratory of Medicinal Chemistry for Natural Resource (Yunnan University), Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, PR China
[b] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission and Ministry of Education, Yunnan University of Nationalities, Kunming 650050, PR China
[c] Institute of Microbiology, Yunnan University, Kunming 650091, PR China

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ABSTRACT

A series of polyhalo isophthalonitrile derivatives (**3** and **4**) that incorporate a variety of substituents at the 2-, 4-, 5- and/or 6-positions of the isophthalonitrile moieties have been designed and synthesized. These derivatives were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus* (Gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria); and *Candida albicans* (Fungi). Compounds **3** and **4** showed stronger inhibition of gram-positive bacteria and fungi growth, and the antimicrobial ability of compound **3j** (a 4-(benzylamino)-5-chloro-2,6-difluoro analog, MIC[SA] = 0.5 μg/mL; MIC[BC] = 0.4 μg/ML; MIC[CA] = 0.5 μg/mL) were close to nofloxacin and fluconazole and identified as the most potent antimicrobial agents in the series. The preliminary analysis of structure–activity relationships is also discussed.

It is well known that halogen plays an important role in living organisms.[1] As an important part of biological activity organic halogenated compounds are widely used as antibacterial, antineoplastic, hormones, pheromones, pesticides.[2] Due to its special character, organic halide plays an important role in drug research. In particular, the antimicrobial activity of organic halide derivatives is also well documented in the literatures,[3,4] for example organic chlorides (Vancomycin, Chlorotetracycline, Chloramphenicol, Calicheamycin, Rebeccamycin, and Ambigol A), bromides (Pyrolnitrin) and iodide (Calicheamicin). Recently, the use of fluorinated compounds as bioactive or functional molecules has increased in pharmaceutical research due to the unique effects of F-substituents in pharmaceutical formulations. Fluorine incorporation into biologically-active compounds can alter drug metabolism or enzyme substrate recognition.[5] The hydrophobic nature of fluorinated compounds has also been cited for its ability to improve transport across the blood–brain barrier. Improved oral bioavailability is seen in some systems where fluorine substitution leads to improved hydrolytic stability. Moreover, polyhalo compounds are also widely present in nature, its anti-microbial activity is attracting more attention. A series of polyhalo compound derivatives have been identified as potent antimicrobial agents.[6,7]

Organic cyanide is halogen isostere and often called virtual analog halogen.[8] The biological compatibility, stability of cyanoacrylate and ability to build relationship plays a key role. In particular, organic cyanide and organic halogenated compounds were expected to have the similar effects of anti-bacterial active. Organic cyanide has an important role in the study of drugs and drug intermediates in the development,[9] cyanoacrylate derivatives can also generate further amine, aldehyde carboxylic acid, and such compounds, and more and more cyanide-containing drugs are being used for clinical treatment. Recently, isophthalonitrile derivatives, one of the most important organic cyanide compounds, have received much attention. They are biologically active. For example, they are inhibitory of HIV-1 reverse transcriptase[10] and mPGES-1,[11] and they have anti-inflammatory[12] and insecticidal activity, etc. Such properties have been explored and a lot of preparation methods of this type of compounds have been developed.[13,14]

Polyhalo isophthalonitriles, especially polyfluoro-isophthalonitrile, have been widely used as anti-cancer,[15] anti-inflammatory[16] and insecticidal[17] agents, among other uses[18] in pharmaceuticals. Our previously works have explored the antitumor and anti-HIV activities of polyhalo isophthalonitriles, and all were found to have good bio-activities.[19–22]

* Corresponding authors. Tel./fax: +86 871 5033215 (J.L.).
E-mail address: linjun@ynu.edu.cn (J. Lin).
† These authors contributed equally to this work.

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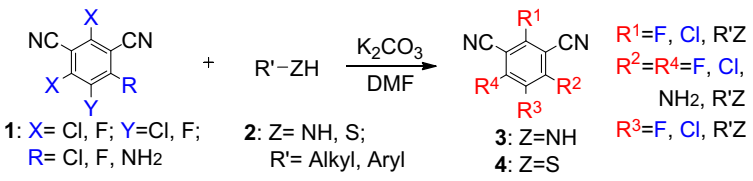

Scheme 1.

Table 1
Zone of inhibition of data for polyhalo isophthalonitrile against different bacteria and fungi at a concentration of 1000 μg/mL

Compound	R^1	R^2	R^3	R^4	Time (h)	Yield (%)	Zone of inhibition (in mm)				
							Gram-positive[a]		Gram-negative[b]		Fungi[c]
							SA	BC	EC	PA	CA
1a	Cl	Cl	Cl	Cl			20.1	20.2	N	N	9.9
1b	F	F	Cl	F			N	N	N	N	N
1c	F	F	F	F			N	N	N	N	N
1d	Cl	NH_2	Cl	Cl	12	98	N	N	N	N	N
1e	F	NH_2	Cl	F	3	85	8.6	7.4	N	N	7.3
1f	F	NH_2	F	F	0.75	81	11.8	8.0	N	7.8	7.5
3a	Cl	Cl	Cl	PhNH	8	95	N	N	N	N	15.3
3b	F	F	Cl	PhNH	1	96	13.0	14.6	N	7.5	13.6
3c	F	F	F	PhNH	0.5	96	23.0	17.8	N	8.0	17.3
3d	Cl	Cl	Cl	*p*-MeOPhNH	8	93	N	N	N	N	N
3e	F	F	Cl	*p*-MeOPhNH	1	95	N	6.8	N	N	N
3f	F	F	F	*p*-MeOPhNH	1	96	8.7	8.6	N	N	N
3g	F	F	Cl	*p*-ClPhNH	10	93	10.7	12.2	N	7.6	N
3h	F	F	F	*p*-ClPhNH	1	95	19.8	16.7	N	7.8	13.3
3i	Cl	Cl	Cl	BnNH	5	92	N	N	N	N	N
3j	F	F	Cl	BnNH	1	95	23.1	22.6	N	N	13.9
3k	F	F	F	BnNH	0.5	93	19.3	18.1	N	N	11.6
3l	F	NH_2	Cl	$Me_2N(CH_2)_3NH$	3	91	14.2	15.0	N	N	–
3m	F	NH_2	F	$Me_2N(CH_2)_3NH$	1	92	7.0	7.1	N	N	–
3n	Cyclohexane	NH_2	Cl	Cyclohexane	8	84	N	N	N	N	–
4a	Cl	Cl	Cl	PhS	16	88	7.3	7.0	N	N	6.8
4b	F	PhS	Cl	PhS	1	61	N	N	N	N	N
4c	PhS	PhS	F	PhS	12	56	N	9.8	N	N	N
4d	F	$CH_3CH_2CH_2S$	Cl	$CH_3CH_2CH_2S$	2	73	8.8	7.5	N	N	N
4e	PhS	NH_2	PhS	PhS	24	52	N	N	N	N	–
4f	Cl	NH_2	Cl	$CH_3CH_2CH_2S$	10	70	7.0	7.0	N	N	–
4g	F	NH_2	Cl	$CH_3CH_2CH_2S$	3	85	8.5	7.6	N	N	–
4h	$CH_3CH_2CH_2S$	NH_2	Cl	$CH_3CH_2CH_2S$	6	83	7.5	N	N	N	–
4i	$CH_3CH_2CH_2S$	NH_2	F	$CH_3CH_2CH_2S$	3	85	7.9	7.4	N	N	–
Norfloxacin							19.9	15.2	31.0	18.0	
Fluconazole											22.5

N-No inhibition zone.
[a] Gram-positive bacteria; BS, *Bacillus cereus* ATCC 14579; SA, *Staphyloccocus aureus* ATCC 6538.
[b] Gram-negative bacteria; EC, *Escherichia coli* ATCC 8099; PA, *Pseudomonas aeruginosa* ATCC 15442.
[c] CA, *Candida albicans* ATCC 10231.

In view of the high degree of bio-activity shown by polyhalo isophthalonitriles derivatives, we have focused on the design of novel structural entities that incorporate both polyhalo and cyanide structural moieties into a single molecular scaffold to evaluate the potential additive effects of these two atoms systems on biological activity, especially with regard to antimicrobial activity. At the same time, the halo-atoms of polyhalo isophthalonitrile can be employed in nucleophilic substitution with nucleophiles. The cyano group can also be modified into an amido or carboxyl group, providing many opportunities for constructing more molecular libraries for biological activity screening.

In the continuation of our investigation on the utility of mild conditions for the synthesis of useful bioactive organic molecules under mild conditions,[16,23] we have utilized K_2CO_3 as an efficient and ecofriendly catalyst in DMF assisted synthesis of a series of amino-isophthalonitrile (**3a**–**3n**) and thio-isophthalonitrile (**4a**–**4i**) derivatives. These compounds were different molecular diversity substitute products. The synthetic routes to these two series of compounds (**3a**–**3n** and **4a**–**4i**) are illustrated in Scheme 1.

This type of reaction features a S_NAr mechanism,[18] R^2 and R^4 were the most active part due to strong electron withdrawing group (halogen and cyano group) on the benzene ring. The desired compounds (**3a**–**3n**) and (**4a**–**4i**) were formed from **2a**–**2n** and **2o**–**2w** in excellent yields: 84–96% and 52–88%, respectively. Comparative data for these compounds with respective to reaction time and yield of product are provided in Table 1. All of the synthesized compounds were characterized by ^1H NMR, ^{13}C NMR and ^{19}F NMR spectrometry and HRMS analysis.[24]

Derivatives (**1a**–**1f**, **3a**–**3n** and **4a**–**4i**) were initially screened for in vitro antibacterial activity against gram-positive bacterial strains (*Staphyloccocus aureus* ATCC 6538 [SA], *Bacillus cereus* ATCC 14579 [BC]), gram-negative bacterial strains (EC, *Escherichia coli* ATCC 8099 [EC], *Pseudomonas aeruginosa* ATCC 15442 [PA]), and fungal strains (*Candida albicans* ATCC 10231 [CA]) utilizing the disk diffusion assay.[25,26] The antibiotic drug, norfloxacin was also used as a positive control. Antibacterial screening for derivatives and the positive control was performed at a fixed concentration of 1000 μg/mL.

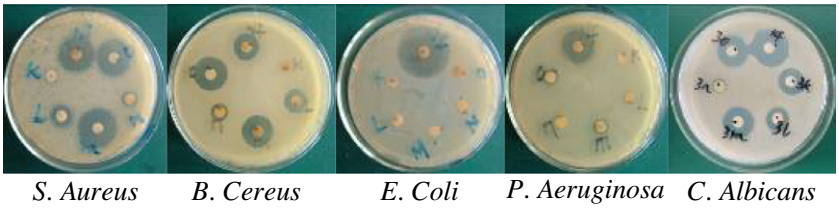

Figure 1. Antibacterial activity of halogenated derivatives of polyhalo isophthalonitrile screening of partial results.

Table 2
Minimum inhibitory concentration values for polyhalo isophthalonitrile against different bacteria and fungi

Compound	Minimum inhibitory concentration[a]		
	Gram-positive[b]		Fungi[c]
	SA	BC	CA
1a	0.5	0.7	0.5
1e	46.9	>1000	15.6
1f	125	104.2	62.5
3b	3.9	125	0.5
3c	3.3	6.8	3.9
3f	375	>1000	n
3g	26	416.7	n
3h	1.5	31.3	2.3
3j	0.5	0.4	0.5
3k	1.3	3.9	10.4
4a	250	>1000	>500
4d	11.7	>1000	>500
4f	500	>1000	>1000
4g	1.6	>1000	>500
4h	250	>1000	>1000
Norfloxacin	0.5–2	0.5–2	—
Fluconazole	—	—	1–3

[a] MIC were determined by microbroth dilution technique and values reported in the table represent the values obtained in triplicate.
[b] Gram-positive bacteria; BS, *Bacillus cereus* ATCC 14579; SA, *Staphyloccocus aureus* ATCC 6538.
[c] CA, *Candida albicans* ATCC 10231.

All of the compounds in Table 1 exhibited different antibacterial activities for all five bacterial strains. Mostly, compounds exhibited antibacterial activity against gram-positive bacterial strains with zones of inhibition (ZOI) ranging to a maximum of 23.1 mm (Fig. 1). However, compounds **1b–1d**, **3a**, **3d**, **3e**, **3i**, **3n**, **4b** and **4e** showed no significant inhibition of growth of bacterial activity to four kinds of bacteria strains. Derivatives **1a**, **1e**, **1f**, **3b**, **3c**, **3f–3h**, **3j**, **3k**, **4a**, **4d**, and **4f–4h** were identified as potent antibacterial agent against gram-positive bacterial strains. 2,4,5,6-tetrachloroisophthalonitrile (**1a**, ZOI[SA] = 20.1 mm, ZOI[BC] = 20.2 mm, ZOI[CA] = 9.9 mm); 2,4,5-trifluoro-6-(phenylamino)isophthalonitrile (**3c**, ZOI[SA] = 23.0 mm, ZOI[BC] = 17.8 mm, ZOI[CA] = 17.3 mm); 4-(benzylamino)-5-chloro-2,6-difluoroisophthalonitrile (**3j**, ZOI[SA] = 23.1 mm, ZOI[BC] = 22.6 mm, ZOI[CA] = 13.9 mm); 4-(benzylamino)-2,5,6-trifluoroisophthalonitrile (**3k**, ZOI[SA] = 23.1 mm, ZOI[BC] = 22.6 mm, ZOI[CA] = 13.9 mm); 5-chloro-2-fluoro-4,6-bis(propylthio)isophthalonitrile (**4d**, ZOI[SA] = 8.8 mm, ZOI[BC] = 7.5 mm), 4-amino-5-chloro-2-fluoro-6-(propylthio)isophthalonitrile (**4g**, ZOI[SA] = 8.5 mm, ZOI[BC] = 7.6 mm) showed good antibacterial activity against all gram-positive bacterial strains. Based on the data from the antibacterial studies against gram-positive bacterial strains, the following observations can be made. Derivatives **3** and **4** mostly exhibited moderate to high antibacterial activity. More importantly, introducing fluorine atom substituents at the benzene rings in a series of isophthalonitrile derivatives can improve their antibacterial activity, and compounds **1a**, **3c**, **3j** and **3k** exhibited similar antibacterial activities as the standard antibiotic drug, norfloxacin. For results of the experiment see Table 1.

Derivatives (**1a**, **1e**, **1f**, **3a–3c**, **3h**, **3j**, **3k and 4a**) were also examined for antifungal activity against fungal strains, that is *Candida albicans* [CA] (Table 1, Fig. 1). The antifungal drug, Fluconazole was used as a positive control. The fungal strains were grown and maintained on Sabouraud glucose agar plates. The plates were incubated at 26 °C for 72 h, and the resulting ZOIs were measured.[27,28] Antifungal screening for derivatives and positive control was performed at a fixed concentration of 1000 μg/mL. 2,4,5-Trichloro-6-(phenylamino)isophthalonitrile (**3a**, ZOI[CA] = 15.3 mm), 5-chloro-2,4-difluoro-6-(phenylamino)isophthalonitrile (**3b**, ZOI[CA] = 13.6 mm), 2,4,5-trifluoro-6-(phenylamino)isophthalonitrile (**3c**, ZOI[CA]=15.3 = 15.3 mm), 4-((4-chlorophenyl)amino)-2,5,6-trifluoroisophthalonitrile (**3h**, ZOI[CA] = 13.3 mm), 4-(benzylamino)-5-chloro-2,6-difluoroisophthalonitrile (**3j**, ZOI[CA] = 13.9 mm), 4-(benzylamino)-2,5,6-trifluoroisophthalonitrile (**3k**,ZOI[CA] = 13.9 mm), 2,4,5-trichloro-6-(phenylthio)isophthalonitrile (**4a**, ZOI[CA] = 6.8 mm) were identified as the most potent antifungal agents against all three fungal strains. Based on the screening data from the antifungal studies, the following observations can be made. Thio-isophthalonitrile (**4a–4i**) derivatives have few effects on fungi, while compounds **3a–3c**, **3h**, **3j** and **3k** have good antifungal activity.

The minimum inhibitory concentration (MIC) values for derivatives (**1a–1f**, **3a–3n** and **4a–4i**) and the positive control drugs norfloxacin and fluconazole were also determined against the two bacterial strains and the one fungal strains by the liquid dilution method.[29,30] Concentrations of derivatives and positive control drugs at 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 μg/mL were prepared in dimethyl sulfxide (DMSO) solution. Inoculums of the bacterial and fungal cultures were also prepared. Inoculum and sterile water were added to a series of tubes each containing 1 mL of test compound solution at the 11 different concentrations. The tubes were incubated for 24 h and carefully observed for the presence of turbidity. The minimum concentration at which no growth was observed was taken as the MIC value. The MIC values for all the derivatives examined ranged from 0.98

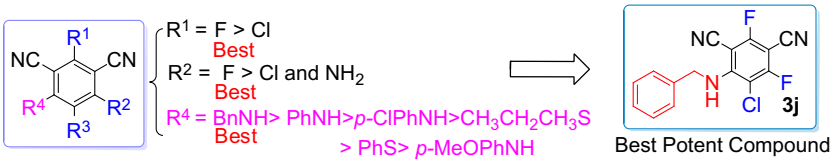

Figure 2. Structure–activity relationship of polyhalo isophthalonitrile.

to 500 μg/mL. Several derivatives exhibited superior antimicrobial activity compared to the positive control drugs, norfloxacin and fluconazole. Three derivatives, 2,4,5,6-tetrachloroisophthalonitrile (**1a**, MIC[SA] = 0.5 μg/mL; MIC[BC] = 0.7 μg/mL), 4-(benzylamino)-5-chloro-2,6-difluoro-isophthalo nitrile (**3j**, MIC[SA] = 0.5 μg/mL; MIC[BC] = 0.4 μg/mL) and 4-(benzylamino)-2,5,6-trifluoroisophthalonitrile (**3k**, MIC[SA] = 1.3 μg/mL; MIC[BC] = 3.9 μg/mL) were identified as potent antibacterial agents against gram-positive bacterial strains; these derivatives showed the same magnitude of antibacterial activity as the standard antibiotic, norfloxacin. Four compounds, **1a**, **3b**, **3h** and **3j**, showed good potency against *Candida albicans* [CA], and the antifungal activities of derivatives **1a, 3b** and **3j** were superior to the antifungal drug fluconazole. However, analog **4** had poor antifungal activity. The MIC data for all of the derivatives against the different bacterial and fungal strains are shown in Table 2.

A comparison of the activities of the polyhalo isophthalonitrile derivatives **1** and **4** suggested that most polyhalo isophthalonitrile derivatives showed good activity against Gram-positive bacteria and antifungal activity, and were selectively anti-microbial. Compounds **1a**, **3c**, **3h**, **3j** and **3k** showed strong antibacterial activity, similar to the positive control drugs. Substitution at the 6-positions with S-substituted groups resulted in lower activity than N-substituted groups, especially with regard to antifungal activity. The number of fluorine atoms in Benzene ring and BnNH group was found to have a positive influence on the antimicrobial properties of polyhalo isophthalonitrile. We speculated that these compounds have effect on the cell wall, which can suspend the growth of the cell membrane, leading to the growth inhibitor or death of bacteria or fungi.

We found that substitution at the 2- and 4-positions with a fluorine atom, and at the 6-position with n-BnNH, plays an important role in regulating antimicrobial and antifungal activity (Fig. 2). This was evident as **3j** and **3k** showed good activity against the microbes and fungi used in our study (Table 2). Compound **3j** proved to be the most promising for further structural modifications guided by the valuable information provided by the detailed SARs described here.

In summary, a series of polyhalo isophthalonitrile derivatives (**3** and **4**) that incorporate a variety of substituents at the 2-, 4-, 5- and/or 6-positions of the isophthalonitrile moieties, have been designed and synthesized. These derivatives (**1**, **3**, and **4**) were evaluated for their antimicrobial activity against gram-positive bacteria, gram-negative bacteria and Fungi strains. Compounds **3** and **4** showed stronger inhibition of gram-positive bacteria and fungi growth, and compound **3j** exhibited the most significant antimicrobial activity, (a 4-(benzylamino)-5-chloro-2,6-difluoro analog, MIC[SA] = 0.5 μg/mL; MIC[BC] = 0.4 μg/ML; MIC[CA] = 0.5 μg/mL, close to nofloxacin and fluconazole), which paves the way to finding promising leads for antimicrobials in the future.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.033.

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24. *Analytical data and yields for the new active compounds: 4-(benzylamino)-2,5,6-trichloroisophthalonitrile (**3i**):* white solid; mp 163–164 °C; IR (KBr) (ν_{max}, cm^{-1}) 3338, 2938, 2226, 1571, 1512, 1200, 1090, 735; ^1H NMR (500 MHz, DMSO-d_6): δ 8.11 (br, 1H, NH), 7.36–7.30 (m, 5H, PhH), 5.05 (m, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 150.5, 141.9, 138.6, 138.3, 128.9, 127.6, 127.0, 119.9, 114.6, 113.9, 101.8, 94.5, 47.6; HRMS (TOF ES$^-$) *m/z* calcd for $C_{15}H_7Cl_3N_3^-$ [(M−H)$^-$], 333.9711; found, 333.9718. *4-(Benzylamino)-5-chloro-2,6-difluoroisophthalonitrile (**3j**):* white solid; mp 106–108 °C; IR (KBr) (ν_{max}, cm^{-1}) 3396, 2231, 1627, 1528, 1458, 1079, 744, 449; ^1H NMR (500 MHz, Acetone-d_6): δ 7.67 (br, 1H, NH), 7.40–7.30 (m, 5H, PhH), 5.17–5.16 (d, *J* = 6.5 Hz, 2H, CH_2); ^{13}C NMR (125 MHz, Acetone-d_6): δ 167.2 (d, *J* = 261.3 Hz), 161.4 (d, *J* = 257.5 Hz), 151.9, 138.8, 130.0, 129.0, 128.4, 112.6, 109.3, 105.8 (d, *J* = 18.8 Hz), 83.5 (d, *J* = 20.0 Hz), 81.9, 48.8; HRMS (TOF ES$^-$) *m/z* calcd for $C_{15}H_7ClF_2N_3^-$ [(M−H)$^-$], 302.0302; found, 302.0294. *4-(Benzylamino)-2,5,6-trifluoroisophthalonitrile (**3k**):* white solid; mp 122–124 °C; IR (KBr) (ν_{max}, cm^{-1}) 3399, 2926, 2230, 1641, 1529, 1295, 1072, 750, 605, 485; ^1H NMR (500 MHz, DMSO-d_6): δ 8.73 (br, 1H, NH), 7.34–7.27 (m, 5H, PhH), 4.84 (m, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.8 (d, *J* = 258.8 Hz), 151.9 (d, *J* = 256.3 Hz), 144.9, 138.5, 136.0 (dd, *J*1 = 241.3 Hz, *J*2 = 11.3 Hz), 128.9, 127.3, 126.9, 111.6, 109.1, 82.3 (d, *J* = 17.5 Hz), 78.6 (t, *J* = 20.0 Hz), 46.9; HRMS (TOF ES$^+$) *m/z* calcd for $C_{15}H_8F_3N_3Na^+$ [(M+Na)$^+$], 310.0563; found, 310.0570. *4-Amino-5-chloro-6-(3-(dimethylamino)propylamino)-2-fluoroisophthalonitrile (**3l**):* white solid; mp 170–171 °C; IR (KBr) (ν_{max}, cm^{-1}) 3343, 2921, 2825, 2217, 1628, 1527, 1468, 868; ^1H NMR (500 MHz, DMSO-d_6): δ 7.94 (br, 1H, NH), δ 6.90–6.88 (br, 2H, NH_2), 3.70–3.31 (m, 2H, CH_2NH), 2.51–2.38 (m, 2H, CH_2N), 2.18 (s, 6H, $2CH_3N$), 1.75 (m, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.3 (d, *J* = 253.8 Hz), 149.1 (d, *J* = 6.3 Hz), 148.9 (d, *J* = 6.3 Hz), 114.0, 112.5, 96.7, 75.5 (d, *J* = 18.8 Hz), 73.5 (d, *J* = 18.8 Hz), 57.8, 45.4, 45.3, 44.7, 26.3; ^{19}F NMR (470 MHz, DMSO-d_6) δ −102.6 (s, 1F); HRMS (TOF ES$^-$) *m/z* calcd for $C_{13}H_{15}ClFN_5^-$ [(M−H)$^-$], 294.0927; found, 294.0977. *4-Amino-6-(3-(dimethylamino)propylamino)-2,5-difluoroisophthalonitrile (**3m**):* white solid; mp 162–164 °C; IR (KBr) (ν_{max}, cm^{-1}) 3355, 2954, 2827, 2220, 1631, 1524, 1291, 569; ^1H NMR (500 MHz, DMSO-d_6): δ 7.41 (br, 1H, NH), δ 6.88 (br, 2H, NH_2), 3.65–3.55 (m, 2H, CH_2NH), 2.39–2.28 (m, 2H, CH_2N), 2.15 (s, 6H, $2CH_3N$), 1.72–1.70 (m, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.4 (d, *J* = 252.5 Hz), 142.2 (q, *J* = 7.5 Hz), 141.5, 133.2 (d, *J* = 226.3 Hz), 113.4, 112.4, 75.0 (d, *J* = 20.0 Hz), 74.3 (d, *J* = 18.8 Hz), 57.4, 45.4, 45.3, 43.8, 27.2; ^{19}F NMR (470 MHz, DMSO-d_6) δ −106.7 (d, *J* = 9.4 Hz, 1F), −159.9 (s, 1F); HRMS (TOF ES$^-$) *m/z* calcd for $C_{13}H_{15}F_2N_5^-$ [(M−H)$^-$], 278.1223; found, 278.1230. *4-Amino-5-chloro-2,6-di(piperidin-1-yl)isophthalonitrile (**3n**):* yellow solid; mp 120–121 °C; IR (KBr) (ν_{max}, cm^{-1}) 3352, 2934, 2851, 2207, 1621, 1551, 1444, 865; ^1H NMR (500 MHz, DMSO-d_6): δ 6.65 (br, 2H, NH_2), 3.38–3.23 (m, 8H, $4CH_2N$), 1.62–1.60 (m, 12H, $6CH_2$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.3, 156.8, 152.9, 117.9, 116.8, 104.7, 89.8, 84.5, 53.1, 53.1, 52.3, 52.3, 31.0, 26.6, 26.6, 26.4, 24.0, 23.9; HRMS (TOF ES$^-$) *m/z* calcd for $C_{18}H_{21}ClN_5^-$ [(M−H)$^-$], 342.1491; found, 342.1499. *2,4,5-Trichloro-6-(phenylthio)isophthalonitrile (**4a**):*

yellow solid; mp: 154–156 °C; IR (KBr) (ν_{max}, cm^{-1}) 3437, 2228, 1527, 1345, 1219, 750, 496; ^1H NMR (500 MHz, DMSO-d_6): δ 7.37–7.17 (m, 5H, PhH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 144.2, 143.5, 140.6, 133.3, 130.6, 130.0, 130.0, 129.6, 129.6, 128.7, 121.6, 114.1; HRMS (TOF ES$^-$): m/z calcd for $C_{14}H_4Cl_3N_2S^-$ [(M–H)$^-$], 336.9166; found, 336.9175. *5-Chloro-2-fluoro-4,6-bis(phenylthio)isophthalonitrile (**4b**):* yellow solid; mp: 170–171 °C; IR (KBr) (ν_{max}, cm^{-1}) 3445, 2230, 1581, 1477, 1338, 1226, 744, 473; ^1H NMR (500 MHz, DMSO-d_6): δ 7.38–7.27 (m, 10H, PhH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 145.4, 143.6, 141.8, 133.6, 133.3, 130.1, 130.1, 130.1, 129.5, 129.1, 129.1, 128.1, 128.1, 126.7, 114.4, 114.4; ^{19}F NMR (470 MHz, DMSO-d_6): δ = –100.7 (s, 1F); HRMS (TOF ES$^-$): m/z calcd for $C_{20}H_9ClFN_2S_2^-$ [(M–H)$^-$], 394.9885; found, 394.9891. *5-Fluoro-2,4,6-tris(phenylthio)isophthalonitrile (**4c**):* yellow solid; mp: 122–124 °C; IR (KBr) (ν_{max}, cm^{-1}) 3436, 2230, 1576, 1477, 1376, 1296, 746, 474; ^1H NMR (500 MHz, DMSO-d_6): δ 7.53–7.20 (m, 15H, PhH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.1 (d, J = 251.3 Hz), 138.6, 133.7, 133.2, 133.1, 132.9, 132.2, 131.6, 130.3, 130.1, 130.0, 129.7, 129.5, 128.9, 128.5, 128.2, 125.4, 114.2, 110.9, 107.2 (d, J = 16.3 Hz); ^{19}F NMR (470 MHz, DMSO-d_6): δ = –88.7 (s, 1F); HRMS (TOF ES$^-$): m/z calcd for $C_{26}H_{14}FN_2S_3^-$ [(M–H)$^-$], 469.0309; found, 469.0319. *5-Chloro-2-fluoro-4,6-bis(propylthio)isophthalonitrile (**4d**):* yellow solid; mp: 60–62 °C; IR (KBr) (ν_{max}, cm^{-1}) 3451, 2967, 2235, 1566, 1394, 1236, 1093, 794; ^1H NMR (500 MHz, DMSO-d_6): δ 3.26–3.13 (m, 4H, $2CH_2S$), 1.58–1.57 (m, 4H, $2CH_2$), 0.96 (m, 6H, $2CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.9 (d, J = 268.8 Hz), 145.5, 145.5, 139.3, 111.6, 111.6, 108.0, 107.9, 38.1, 38.1, 23.1, 23.1, 13.1, 13.1; ^{19}F NMR (470 MHz, DMSO-d_6): δ = –100.3 (s, 1F); HRMS (TOF ES$^-$): m/z calcd for $C_{14}H_{13}ClFN_2S_2^-$ [(M–H)$^-$], 327.0198; found, 327.0209. *4-Amino-2,5,6-tris(phenylthio)isophthalonitrile (**4e**):* white solid; mp 249–250 °C; IR (KBr) (ν_{max}, cm^{-1}) 3309, 2225, 1624, 1529, 746, 470; ^1H NMR (500 MHz, DMSO-d_6): δ 7.52–7.14 (m, 17H, PhH, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.1, 150.8, 144.7, 135.2, 134.3, 133.8, 130.0, 129.8, 129.8, 129.5, 129.1, 127.9, 127.4, 127.1, 126.8, 122.2, 115.6, 115.0, 110.6, 102.1; HRMS (TOF ES$^-$) m/z calcd for $C_{26}H_{16}N_3S_3^-$ [(M–H)$^-$], 466.0512; found, 466.0520. *4-Amino-2,5-dichloro-6-(propylthio)isophthalonitrile (**4f**):* white solid; mp 121–123 °C; IR (KBr) (ν_{max}, cm^{-1}) 3327, 2964, 2227, 1637, 1556, 1211, 726, 528; ^1H NMR (500 MHz, DMSO-d_6): δ 7.67–7.62 (br, 2H, NH_2), 3.10–3.04 (m, 2H, CH_2S), 1.57–1.48 (m, 2H, CH_2), 0.98–0.82 (m, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 151.4, 143.1, 139.6, 123.0, 115.3, 113.8, 105.2, 96.4, 37.7, 23.0, 13.2; HRMS (TOF ES$^-$) m/z calcd for $C_{11}H_8Cl_2N_3S^-$ [(M–H)$^-$], 283.9821; found, 283.9828. *4-Amino-5-chloro-2-fluoro-6-(propylthio)isophthalonitrile (**4g**):* white solid; mp 155–156 °C; IR (KBr) (ν_{max}, cm^{-1}) 3324, 2965, 2232, 1635, 1593, 1239, 787; ^1H NMR (500 MHz, DMSO-d_6): δ 7.79–7.68 (br, 2H, NH_2), 3.09–2.88 (m, 2H, CH_2S), 1.56–1.48 (m, 2H, CH_2), 0.97–0.81 (m, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.5 (d, J = 261.3 Hz), 152.4 (d, J = 5.0 Hz), 143.6, 120.6, 113.7, 112.3, 93.4 (d, J = 13.8 Hz), 85.9 (d, J = 18.8 Hz), 38.4, 23.9, 14.0; ^{19}F NMR (470 MHz, DMSO-d_6) δ –102.2 (s, 1F); HRMS (TOF ES$^-$) m/z calcd for $C_{11}H_8ClFN_3S^-$ [(M–H)$^-$], 268.0117; found, 268.0127. *4-Amino-5-chloro-2,6-bis(propylthio)isophthalonitrile (**4h**):* white solid; mp 106–107 °C; IR (KBr) (ν_{max}, cm^{-1}) 3338, 2963, 2222, 1622, 1545, 1203, 724; ^1H NMR (500 MHz, DMSO-d_6): δ 7.53 (br, 2H, NH_2), 3.08–3.02 (m, 4H, $2CH_2S$), 1.54–1.47 (m, 4H, $2CH_2$), 0.97–0.92 (m, 6H, $2CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 151.0, 142.9, 142.7, 124.1, 116.4, 115.0, 111.0, 101.7, 38.3, 37.6, 23.0, 22.9, 13.2, 13.2; HRMS (TOF ES$^-$) m/z calcd for $C_{14}H_{15}ClN_3S_2^-$ [(M–H)$^-$], 324.0401; found, 324.0409. *4-Amino-5-fluoro-2,6-bis(propylthio)isophthalonitrile (**4i**):* yellow solid; mp 81–82 °C; IR (KBr) (ν_{max}, cm^{-1}) 3344, 2966, 2219, 1631, 1472, 1273, 606; ^1H NMR (500 MHz, DMSO-d_6): δ 7.38 (br, 2H, NH_2), 3.05–3.01 (m, 4H, $2CH_2S$), 1.56–1.48 (m, 4H, $2CH_2$), 0.98–0.93 (m, 6H, $2CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 150.0 (d, J = 242.5 Hz), 144.6 (d, J = 17.5 Hz), 139.9, 129.7 (d, J = 17.5 Hz), 116.0, 114.8, 108.3, 101.8, 38.4, 36.7, 23.0, 22.9, 13.1, 13.1; HRMS (TOF ES$^-$) m/z calcd for $C_{14}H_{15}FN_3S_2^-$ [(M–H)$^-$], 308.0697; found, 308.0706.

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