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Design, synthesis, and pharmacological evaluation of some 2-[4-morpholino]-3-aryl-5-substituted thiophenes as novel anti-inflammatory agents: generation of a novel anti-inflammatory pharmacophore

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Abstract—Compounds incorporating a thiophene moiety, a pi excess five membered heterocycle, have attracted a great deal of research interest owing to the therapeutic utility of the template as useful drug molecular scaffolding. Recently we reported the anti-inflammatory activity profile exhibited by two thiophene analogs, AP84 and AP82 in acute and chronic models of inflammation. The good activity profile exhibited by AP84, a 3-(substituted aryl)-2-(4-morpholino)-5-heteroaryl substituted analog of thiophene, in the formalin induced rat paw edema chronic model as compared to a weak activity in acute carrageenin induced rat paw edema, and the slightly better protection exhibited in the acute model by AP82 (27%), the 5-aroyl analog provided an impetus for a proper exploration of their structural types. In this paper we report the synthesis and pharmacological evaluation of some novel, 2-(4-morpholino)-3-(substituted aryl)-5-substituted thiophenes, as possible anti-inflammatory leads. The 3-(4-chlorophenyl)-2-(4-morpholino) thiophene analogs AP49, AP158, and AP88 provided a protection of 20%, 23%, and 20%, respectively, when screened for anti-inflammatory activity in carrageenin induced rat paw edema, an acute in vivo model, comparable to that of AP82, at a dose level of 100 mg/kg body weight p.o. compared to ibuprofen as standard. The replacement of the 3-(4-chlorophenyl) moiety with the 3-phenyl moiety gave rise to AP50 (30%), AP159 (38%), AP27 (0%), and AP92 (38%), with three analogs being more active in the acute model. Alteration of the group *para* to the phenyl ring at third position, from chloro, to methyl mercapto gave rise to the 3-(4-methylmercapto-phenyl) analogs AP54 (20%), AP160 (0%), and AP73 (52%), with only one analog appearing to be better than AP82. These results indicate that 4-methane sulfonyl aroyl group at 5-position and other substituents of different quadrants of Craig plot on the phenyl moiety at the third position could lead to more potent candidates. However, alteration of aroyl to substituted pyridyl at 5-position with a phenyl group at the third position as in AP26 gave rise to much better protection (66%) again reinforcing the importance of the heteroaryl ring at the fifth position and implying its utility in the composition of a novel pharmacophore for designing better trisubstituted thiophenes as anti-inflammatory agents.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain, fever, and inflammatory conditions including osteoarthritis. In an effort to develop novel drug designing strategies in anti-inflammatory research, we came up with a molecule AP84;[1] Figure 1, which we proposed, could have a possible dual inhibitory action along the inflammatory cascade. Even though the molecule exhibited weak activity in acute anti-inflammatory screening, and gave only 17% protection in carrageenin induced rat paw edema model, in the

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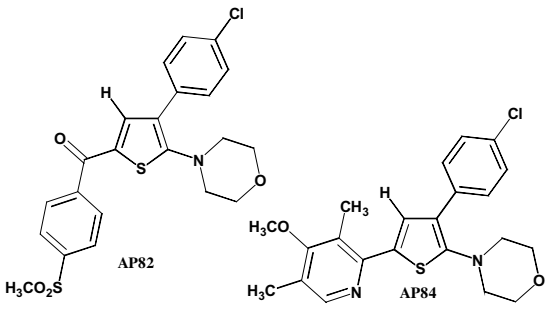


Figure 1. Trisubstituted thiophene AP82 and AP84 designed and synthesized as anti-inflammatory agents. AP82 had an aroyl substitution at the fifth position of the thiophene and AP84 had a pyridyl substitution at the fifth position.

chronic model of the formalin induced paw edema it gave protection of 54% on day one and 77% on day five and had a similar profile to dexamethasone in this model. On the other hand its counterpart AP82, with an aroyl substitution at the fifth position of the thiophene gave better protection (27%) in the acute model but not in the chronic model. We wanted to probe into this outcome, as this seemed unusual. Hence, we synthesized a series of trisubstituted thiophenes with incorporation of aroyl substitution at the fifth position with an N-(4-morpholino) group at second position as in AP82, with substituents on the 3-aryl group of the thiophene. A few analogs with substituted 2-pyridyl moiety at 5-position were also made for anti-inflammatory evaluation to assess its utility.

In this report, we describe the synthesis of a series of 2-morpholino thiophene derivatives as possible leads for anti-inflammatory research. In an attempt to study the structure–activity relationship of these trisubstituted thiophenes we could propose a novel pharmacophore for designing better anti-inflammatory agents laying down the optimum requirements at the third and fifth position of the trisubstituted thiophene analogs with a conformationally restricted morpholine at the second position, synthesized for eliciting better anti-inflammatory activity.

2. Results and discussion

The anti-inflammatory activity exhibited by the three compounds AP49 (20%), AP158 (23%), and AP88 (20%) was not significantly better than that of AP82 (27%) in the acute anti-inflammatory screening model. The (4-chlorophenyl) substitution at the third position of the thiophene was not helping the candidates to improve the pharmacological profile. The third position of thiophene was substituted with 4-thiomethyl phenyl group where thiomethyl is not as electron withdrawing ($\sigma p = 0.00$) as chlorine ($\sigma p = 0.23$). However it can reversibly undergo metabolic oxidation to sulfoxide ($\sigma p = 0.49$) and subsequently irreversible oxidation to sulfone ($\sigma p = 0.72$). The only compound, which was superior to AP82 was AP73, wherein a sulfone was attached to the benzene ring at the fifth position of the thiophene. The biological activities of various analogs were AP54 (20%), AP160 (0%), and AP73 (52%).

In order to clarify the structural requirement for these 2-morpholino derivatives, we synthesized some 2-morpholino derivatives with phenyl ring at the third position of the thiophene with other substituents being the same. The resulting compounds AP50 (30%), AP159 (38%), AP27 (0%), and AP92 (38%) were comparable to the above series or marginally better in eliciting the anti-inflammatory effect.

One plausible reason for the lesser activity shown by AP27 could be that, it undergoes an aromatic hydroxylation followed by a glucoronide conjugation to get eliminated rapidly compared to its counterparts and also this is not possible in 3-(4-thiomethyl phenyl) and 3-(4-chloro phenyl) analogs of thiophene since the *para*-position of the phenyl is blocked.

From the SAR studies it appears that the phenyl series has a marginal edge over all the compounds except AP27 in exhibiting a better biological profile compared to AP82. To delineate the importance of the phenyl ring at the third position and the ketone bridge at the fifth position toward the biological activity, further studies were done. The fact that the counter part of AP84 that is, AP26 was the most active compound in the whole series with phenyl substitution at the third position of the thiophene, give a clue that a ketone bridge might not be preferred at the fifth position as compared to pyridyl (heteroaryl moiety). The morpholine at the second position, the phenyl ring at the third position and a heterocyclic ring like pyridyl at the fifth position can be considered as a good pharmacophore for designing better anti-inflammatory agents.

The fact that AP26 like AP84 neither had the features of a COX-1 inhibitory (ester/acid as in mefenamic acid) nor a COX-2 inhibitory pharmacophore of two adjacent aromatic or heteroaromatic moieties helps in the delineation of a novel structural feature to identify developable drug like candidates to treat conditions of inflammations. If this is true, we see a novel class of compounds, which should act somewhere other than COX-1 or COX-2 inhibition along the inflammatory cascade to avoid the serious side effects implicated by COX-1 and COX-2 inhibitors clinically.

3. Conclusion

A series of 2-morpholino thiophene derivatives were synthesized, characterized and evaluated for their anti-inflammatory activity in carrageenin induced rat paw edema model—an acute in vivo model. The 4-chlorophenyl, 4-thiomethyl phenyl, and phenyl substitution at the third position of the thiophene were evaluated for their modulation of pharmacological activity. From the SAR studies it appears that the phenyl series has a marginal edge over all the compounds. The fact that the counter part of AP84 that is, AP26 was the most active compound in the whole series with phenyl substi-

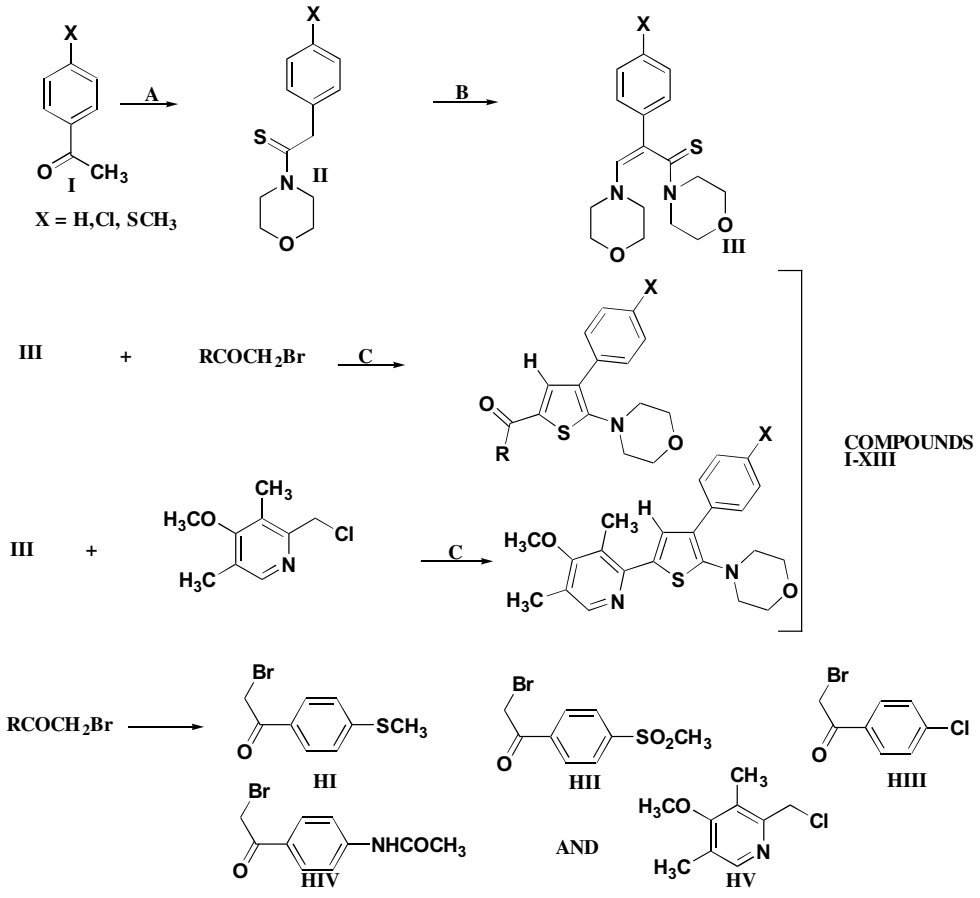
tution at the third position of the thiophene, give a clue that a ketone bridge might not be preferred at the fifth position as compared to pyridyl (heteroaryl moiety). The structure–activity relationship studies explore morpholine at the second position, the phenyl ring at the third position, and a heterocyclic ring like pyridyl at the fifth position as a good pharmacophore for designing better anti-inflammatory agents. The results disclose a new class of anti-inflammatory agents designed and synthesized for the first time wherein the utility of a pyridyl function at the fifth position of the thiophene is reconfirmed. If this were true then it is worth considering this novel pharmacophore in designing new experimental candidates and to further explore their full potential particularly in inflammatory diseases.

4. Experimental

α-Haloketones (HI-HV) were synthesized using reported procedure.[2] 2-Chloromethyl-3,5-dimethyl-4-methoxy-pyridine (HV), was a gift sample from Dishman Pharmaceuticals Ltd. Carrageenin was purchased from Qualigen Mumbai. Ibuprofen was a gift sample from AVIK Pharmaceuticals Ltd Mumbai.

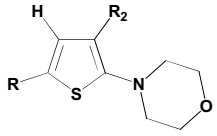
Synthesis of trisubstituted thiophenes: The synthetic sequence (Scheme 1) we utilized for preparing thiophene analogs, were analogs to the method reported in the literature.[3] The reaction was brought about by reacting equimolar amount of thioacrylic acid morpholide intermediate (III) with α-halo carbonyl compound (HI-HIV) or halomethylene compound HV to yield trisubstituted thiophenes (I–XIII) (Table 1).

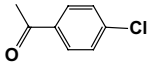
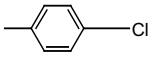
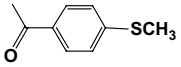
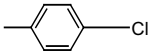
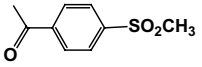
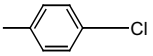
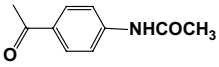
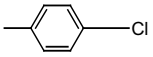
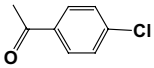
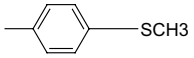
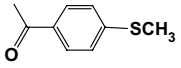
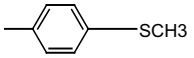
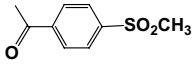
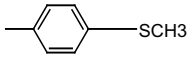
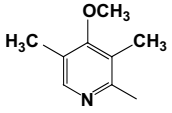
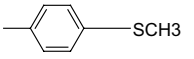
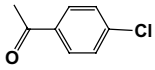
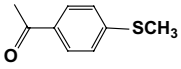
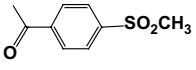
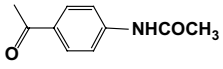
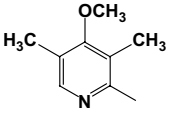
Carrageenin induced rat paw edema:[4] Sprague–Dawley (male/female) rats weighing 150–250 g were used for the edema test. Animals were divided into six rats per group. Rats were put on fast for 18 h prior to the experiment. The standard drug, ibuprofen (100 mg/kg body weight) and the test drugs (100 mg/kg body weight) were given orally as a suspension, in 0.1% sodium CMC as vehicle (Table 2). One hour later, 0.1 mL of 1% carrageenin solution in saline was injected in the sub plantar region of the right hind paw of each rat. After 3 h of the carrageenin injection, the reduction in the paw volume compared to vehicle control was measured using plethysmometer. The institutional ethics committee, constituted by the Ministry of Social Justice and Empowerment, Government of India, approved the experimental protocol.



Scheme 1. Synthesis of trisubstituted thiophenes. The adduct III was reacted with various haloketones of the formula (HI-HIV) and 2-chloromethyl-3,5-dimethyl-4-methoxy-pyridine (HV) to yield the desired products of the formula (I–XIII).

Table 1. Structure of trisubstituted thiophenes designed and synthesized



Experimental candidate	R	R_2
AP49 (I)		
AP158 (II)		
AP82[1] (III)		
AP88 (IV)		
AP54 (V)		
AP160 (VI)		
AP73 (VII)		
AP75 (VIII)		
AP50 (IX)		
AP159 (X)		
AP27 (XI)		
AP92 (XII)		
AP26 (XIII)		

All the synthesized analogs were characterized by IR, ^{1}H NMR, and mass spectra.

4.1. Physical properties of novel synthesized trisubstituted thiophenes (I–XIII)

4.1.1. 5-(*p*-Chloro benzoyl)-2-(4-morpholino)-3-*p*-chloro phenyl-thiophene. AP49 (I). Molecular formula $C_{21}H_{17}Cl_2NO_2S$, MW 417, mp 202–3 °C, R_f = 0.76 mobile phase toluene–acetonitrile (7:3), % yield—66, IR (KBr, cm^{-1}) = 1632 (C=O stretching at fifth position), 1312 (C–N cyclic amine), mass-(LC–MS)—M+1 at m/z = 418, ^{1}H NMR ($CDCl_3$, δ, ppm) = 3.04–3.09 (m, 4H), 3.74–3.78 (m, 4H), 7.2–7.7 (m, 9H).

Table 2. A and B represent the paw volume and the % protection given by the standard and test drugs in carrageenin induced rat paw edema, an acute in vivo model for screening anti-inflammatory drugs

Experimental candidate	A (B)
Control	2.25 ± 0.08
Ibuprofen	0.89 ± 0.13 (60)
AP49 (I)	1.80 ± 0.19 (20)
AP158 (II)	1.73 ± 0.14 (23)
AP82 (III)	1.64 ± 0.11 (27)
AP88 (IV)	1.80 ± 0.14 (20)
AP54 (V)	1.80 ± 0.17 (20)
AP160 (VI)	2.30 ± 0.14 (0)
AP73 (VII)	1.08 ± 0.02 (52)
AP75 (VIII)	2.55 ± 0.10 (0)
AP50 (IX)	1.57 ± 0.12 (30)
AP159 (X)	1.40 ± 0.01 (38)
AP27 (XI)	2.45 ± 0.11 (0)
AP92 (XII)	1.40 ± 0.18 (38)
AP26 (XIII)	0.77 ± 0.18 (66)

The test drugs were dosed at 100 mg/kg body weight p.o. The standard used was ibuprofen, which was dosed at 100 mg/kg body weight.

4.1.2. 5-(*p*-Methyl mercapto benzoyl)-2-(4-morpholino)-3-*p*-chloro phenyl-thiophene. AP158 (II). Molecular formula $C_{22}H_{20}ClNO_2S_2$, MW 429.5, mp 165–66 °C, R_f = 0.79 mobile phase toluene–acetonitrile (7:3), % yield—99%, IR (KBr, cm^{-1}) = 1608 (C=O stretching at fifth position), mass-(LC–MS)—M+1 at m/z = 430, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.5 (s, 3H), 3.04–3.07 (d, 4H), 3.74–3.77 (m, 4H), 7.2–7.4 (m, 4H), 7.50–7.55 (m, 3H), 7.77 (d, 2H).

4.1.3. 5-(*p*-Methyl sulfonyl benzoyl)-2-(4-morpholino)-3-*p*-chloro phenyl-thiophene. AP82 (III). Molecular formula $C_{22}H_{20}ClNO_4S_2$, MW 461.5, mp 222–23 °C, R_f = 0.43 mobile phase toluene–acetonitrile (7:3), % yield—68%, IR (KBr, cm^{-1}) = 1600 (C=O stretching of ketone), 1352 (S(=O) asymmetrical stretching), 1276 (C–N cyclic amine), 1144 (S=(O), symmetrical stretching), mass-(LC–MS)—M+1 at m/z = 463, ^{1}H NMR ($CDCl_3$, δ, ppm) = 3.08–3.11 (s, 8H), 3.75–3.79 (s, 3H), 7.2–7.55 (m, 5H), 7.94–8.08 (m, 4H).

4.1.4. 5-(*p*-Acetyl amino benzoyl)-2-(4-morpholino)-3-*p*-chloro phenyl-thiophene. AP88 (IV). Molecular formula $C_{23}H_{21}ClN_2O_3S$, MW 440.5, mp 148–49 °C, R_f = 0.67 mobile phase toluene–acetonitrile (7:3), % yield—54%, IR (KBr, cm^{-1}) = 3230 (aryl NH stretching), 1724 (C=O stretching), 1624 (C=O stretching of amide), 1558 (NH bending of amide), mass-(LC–MS)—M+1 at m/z = 441, M+2 at m/z = 443, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.225 (s, 3H), 3.0–3.07 (d, 4H), 3.7–3.78 (d, 4H), 7.2–7.6 (m, 9H), 7.8–7.83 (s, 1H).

4.1.5. 5-(*p*-Chloro benzoyl)-2-(4-morpholino)-3-*p*-methyl-thio phenyl-thiophene. AP54 (V). Molecular formula $C_{22}H_{20}ClNO_2S$, MW 429, mp 153 °C, R_f = 0.54 mobile phase toluene–acetonitrile (7:3), % yield—62.5, IR (KBr, cm^{-1}) = 1632 (C=O stretching at fifth position),

mass-(LC–MS)—M+1 at $m/z = 430$, M+2 at $m/z = 432$, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.5 (s, 3H), 3.06–3.10 (m, 4H), 3.74–3.78 (m, 4H), 7.2 (m, 9H).

4.1.6. 5-(*p*-Methyl mercapto benzoyl)-2-(4-morpholino)-3-*p*-methylthio phenyl-thiophene. AP160 (VI). Molecular formula $C_{22}H_{23}NO_2S_3$, MW 430, mp 56–57 °C, $R_f = 0.64$ mobile phase toluene–acetonitrile (7:3), % yield—29, IR (KBr, cm^{-1}) = 1604 (C=O stretching at fifth position), mass-(LC–MS)—M+1 at $m/z = 431$, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.50–2.53 (br s, 6H), 3.05–3.09 (m, 4H), 3.74–3.78 (m, 4H), 7.23–7.73 (m, 7H), 7.77 (d, 2H).

4.1.7. 5-(*p*-Methyl sulfonyl benzoyl)-2-(4-morpholino)-3-*p*-methylthio phenyl-thiophene. AP73 (VII). Molecular formula $C_{23}H_{23}NO_4S_3$, MW 473, mp 210–11 °C, $R_f = 0.6$ mobile phase toluene: methanol (7:3), % yield—78, IR (KBr, cm^{-1}) = 1608 (C=O stretching of ketone), 1304 (S(=O) asymmetrical stretching), 1156 (S=(O), symmetrical stretching), mass-(LC–MS)—M+1 at $m/z = 474$, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.5 (s, 3H), 3.1–3.2 (m, 7H), 3.74–3.79 (m, 4H), 7.24–7.28 (m, 9H).

4.1.8. 5-(3,5-Dimethyl-4-methoxy-2-pyridyl)-2-(4-morpholino)-3-*p*-thiomethyl phenyl-thiophene. AP75 (VIII). Molecular formula $C_{23}H_{26}N_2O_2S_2$, MW 426, mp 140–41 °C, $R_f = 0.7$ mobile phase toluene–acetonitrile (6:4), % yield—30, IR (KBr, cm^{-1}) = 2976 (aliphatic CH stretching), 2850 (aliphatic CH stretching), 1644 (C=C aromatic stretching), 1604 (C=C aromatic stretching), mass-(LC–MS)—M+1 at $m/z = 427$, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.23 (s, 3H), 2.25 (s, 3H), 2.5 (s, 3H), 3.0 (s, 3H), 3.05–3.08 (d, 4H, morpholine), 3.75–3.78 (d, 4H, morpholine), 7.2–7.8 (m, 6H).

4.1.9. 5-(*p*-Chloro benzoyl)-2-(4-morpholino)-3-phenyl-thiophene. AP50 (IX). Molecular formula $C_{21}H_{18}ClNO_2S$, MW 383, 152–54 °C, $R_f = 0.8$ mobile phase toluene–acetonitrile (7:3), % yield—56, IR (KBr, cm^{-1}) = 1624 (C=O stretching at fifth position), mass-(LC–MS)—M+1 at $m/z = 384$, M+2 = 386, ^{1}H NMR ($CDCl_3$, δ, ppm) = 3.0–3.1 (m, 4H), 3.73–3.77 (m, 4H), 7.24–7.78 (m, 10H).

4.1.10. 5-(*p*-Methyl mercapto benzoyl)-2-(4-morpholino)-3-phenyl-thiophene. AP159 (X). Molecular formula $C_{22}H_{21}NO_2S_2$, MW 395, mp 131–32 °C, $R_f = 0.86$ mobile phase toluene–acetonitrile (7:3), % yield—46, IR (KBr, cm^{-1}) = 1604 (C=O stretching at fifth position), 1288 (C–N cyclic amine), mass-(LC–MS)—M+1 at $m/z = 396$, M+Na = 417.9, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.5 (s, 3H), 3.04–3.09 (m, 4H), 3.7–3.77 (m, 4H), 7.26–7.79 (m, 10H).

4.1.11. 5-(*p*-Methyl sulfonyl benzoyl)-2-(4-morpholino)-3-phenyl-thiophene. AP27 (XI). Molecular formula $C_{22}H_{21}NO_4S_2$, MW 427.6, mp 220–22 °C, $R_f = 0.43$ mobile phase toluene–acetonitrile (7:3), % yield—85, IR (KBr, cm^{-1}) = 1620 (C=O stretching of ketone), 1352 (S(=O) asymmetrical stretching), 1300 (C–N cyclic amine), 1168 (S=(O), symmetrical stretching), mass-(LC–MS)—M+1 at $m/z = 428$, ^{1}H NMR ($CDCl_3$, δ, ppm) = 3.1–3.13 (br s, 3H, morpholine and 3H SO_2CH_3), 3.27–3.29 (br s, 1H, morpholine) 3.75–3.78 (br s, 3H), 4.02–4.05 (s, 1H), 7.2–7.55 (m, 6H), 7.94–7.97 (d, 2H), 8.05–8.08 (d, 2H).

4.1.12. 5-(*p*-Acetyl amino benzoyl)-2-(4-morpholino)-3-phenyl-thiophene. AP92 (XII). Molecular formula $C_{23}H_{22}N_2O_3S$, MW 406, mp 215–16 °C, $R_f = 0.46$ mobile phase toluene–acetonitrile (7:3), % yield—90, IR (KBr, cm^{-1}) = 3250 (aryl NH stretching), 1712 (C=O stretching of ketone), 1612 (C=O stretching of amide), 1540 (NH bending of amide), mass-(LC–MS)—M+1 at $m/z = 407$, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.8 (s, 3H), 3.73–3.8 (m, 4H), 3.9–4.0 (m, 4H), 7.2–7.8 (m, 10H), 9.7 (s, 1H).

4.1.13. 5-(3,5-Dimethyl-4-methoxy-2-pyridyl)-2-(4-morpholino)-3-phenyl-thiophene. AP26 (XIII). Molecular formula $C_{22}H_{24}N_2O_2S$, 42%, MW 380.6, mp 136–37 °C, $R_f = 0.2$ mobile phase benzene–methanol (7:3), % yield—42, IR (KBr, cm^{-1}) = 2940 (aliphatic CH stretching), 2850, 1580, 1476, 1432, 1224, 1100, 1000, 988, mass-(LC–MS)—M+1 at $m/z = 381$, ^{1}H NMR ($CDCl_3$, δ, ppm) = 2.25 (s, 3H), 2.492 (s, 3H), 2.9–3.02 (br s, 3H), 3.75–3.79 (m, 8H), 7.25–7.75 (m, 5H), 8.2 (s, 1H).

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References and notes

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